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THE AMERICAN JOURNAL
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ORIGINAL ARTICLES

STUDIES ON THE TRANSMISSIBILITY OF MALARIA BY PLASMA TRANSFUSIONS*

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It has been demonstrated repeatedly[5,6,7,15] that human malaria may be transmitted by transfusions of the whole blood of individuals who have not suffered from clinical malaria for many years. The actual plasmodia have been demonstrated microscopically in the blood of such donors.[6,7,15] It has also been observed that the plasmodia causing human malaria will remain infective in whole blood preserved in the liquid state up to 8 days[1,2] and that animal malaria may be transmitted by whole blood frozen quickly at very low temperatures.[4,8] In view of the rapidly increasing use of transfusions of plasma preserved in the liquid, frozen and dried states, and of the large number of volunteer blood donors, some of whom may have had unrecognized malaria, participating in the plasma program for the Army and Navy, it became of considerable importance to determine the likelihood of transmission of malaria by means of plasma transfusions. Studies therefore were undertaken with this object in view, and it is the purpose of this communication to report the results of 35 administrations of plasma prepared from donors with active malaria, and preserved by different techniques for varying lengths of time.

Methods. The patients who served as donors for the blood to be processed into plasma and as the recipients of the plasma were on the wards of the St. Elizabeth's Hospital, Washington, D. C.† The donors were patients with active therapeutic quartan and estivo-autumnal malaria. The 35 recipients

* This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

† The authors acknowledge with thanks the generous coöperation of Dr. W. Overholser, Superintendent and Dr. W. Eldridge, Principal Medical Officer of St. Elizabeth's Hospital, in making these studies possible.

were patients with general paresis or other central nervous system disease in which malaria was either indicated or not contraindicated.

Two hundred and fifty ml. of blood were drawn from each donor on the dates indicated in Table 1, into 35 ml. of 2.5% sodium citrate using a "closed-vacuum" system. "Thick" smears were made on the capillary blood of each donor at this time. On the same day on which the blood was drawn it was centrifuged at 2500 r.p.m. for 1 hour and then stored overnight at 8° C. On the following morning, the plasma was aspirated without filtration by means of a "closed-vacuum" system with scrupulously aseptic technique. No bacteriostatic was added and when the conditions of the protocol permitted it, bacteriologic cultures were done. Occasionally the plasma from 2 or 3 donors was pooled; usually it was not.

In view of the fact that most of the plasma being used by the armed services at present is dried from the frozen state, it was decided to study first the effects of freezing, since if it were found that freezing reduced the likelihood of transmission, the additional step of drying would prove unnecessary. Accordingly, in 20 instances, the plasma was "shell" frozen immediately after aspiration by horizontal rotation of the bottle in a bath of solid carbon dioxide and alcohol and then stored in the frozen state at −20° C. in a mechanical refrigerator. In 3 of the observations the plasma was dried from the frozen state by placing it in series with a condenser cooled by solid carbon dioxide and a high vacuum pump. When, as will be seen in Table 1, no transmissions were observed following the administration of frozen or dried plasma, the question arose as to whether plasma ever contained enough red cells to transmit malaria. To answer this, 12 administrations of plasma preserved in the liquid state from 1 day to 2 weeks were observed; this preservation was at room temperature averaging 20° to 25° C. When frozen plasma was to be administered, it was thawed in a water-bath at 37° C. on the morning of administration. When dried plasma was to be administered it was restored to the liquid state with an equivalent amount of sterile pyrogen-free distilled water immediately before administration. Administration of all plasma was by a standard intravenous set in which was incorporated a glass tape filter of pore size equivalent to a 150-mesh stainless steel filter for removal of fibrin shreds.

Control "thick" smears were made and examined on each recipient before injection. Despite this precaution, 2 of the recipients (Nos. 25 and 26) were discovered subsequently to have suffered malaria 5 and 10 years previously respectively. All injections were made intravenously. All recipients were followed by taking rectal temperatures twice daily and examining "thick" smears of capillary blood 3 times weekly for at least 9 weeks. This period was selected on the basis of Boyd's observations[3] on 38 cases of quartan malaria artificially induced by whole blood, in whom the mean incubation period before detection of parasites was 9.5 days and in whom 37 of the 38 showed parasites in less than 30 days. All blood smears were stained with diluted Giemsa's stain and examined microscopically under oil by 2 observers.

Results. Thirty-five administrations of plasma prepared from patients with active malaria were made in dosages varying from 60 to 270 ml. No immediate untoward reaction was observed in any instance. A summary of the data concerning the administrations and the result of each is presented in Table 1.

It will be observed that in the 20 administrations of frozen and the 3 of dried plasma, no transmissions of malaria were observed. On the other hand, in the 2 administrations of plasma preserved in the liquid state for 1 day before injection, there was 1 definite transmission and 1 probable transmission, questionable only because the patient had had malaria 5 years previously. In the 5 administrations of plasma preserved in the liquid state for 1 week before injection, no transmissions

were observed except for 1 doubtful case who showed parasites on only one occasion, had no symptoms and turned out to have had malaria 10 years previously. In the 5 administrations of plasma preserved in the liquid state for 2 weeks, no transmissions were observed.

Discussion. It appears from the above findings that the danger of transmission of malaria by any plasma program, regardless of the type of preservation used, is minimal. The only definite transmission observed was caused by liquid plasma 1 day old. Since administration of plasma under 10 days old is performed rarely for reasons of bacteriologic control, it is evident that liquid plasma over 1 week old, frozen and dried plasma are all equally safe as regards malaria transmissibility.

The transmission of human malaria by whole blood preserved up to 8 days[1,2] and of animal malaria by frozen whole blood[4,8] has been reported. It is probable that the reasons for such transmission using whole blood and the lack of transmission using plasma are, first and probably more important, the question of the size of the infecting dose, and second the hemolytic effect of freezing upon the red cell. These reasons would depend upon the assumptions that the vector in this type of malaria transmission was chiefly the parasitized red cell and that no ultramicroscopic form of the plasmodium existed which had thus far escaped detection by the microscopist. The findings in the present study give weight to the validity of these assumptions. It is of interest in this regard that Whitby in a recent review on transfusions remarks that "filtration of plasma or serum offers a complete protection" from the possibility of transmission of malaria.[10] It is assumed that a bacterial filter is meant, and it is regretted that no experimental data are presented. In the present study, no filtration was used in the preparation of the plasma and only a coarse filter sufficient to remove fibrin shreds was used in the administration. With this technique red cells may be detected microscopically in a hanging drop of fresh liquid plasma and red cell "shadows" in a hanging drop of stored liquid or frozen or dried plasma. In connection with the speculation that the parasitized red cell is the probable vector in the transmission, it is of interest to recall the observations made some years ago that in the transmission of therapeutic malaria by small doses of whole blood the injection of incompatible blood resulted in fewer "takes" and longer incubation periods than that of compatible blood.[9,13]

The behavior of the malarial parasite with respect to varying forms of preservation would seem to resemble qualitatively that which has been described by others for the *Treponema pallidum*, which survives preservation in liquid whole blood for 48 hours,[12] and in plasma frozen at −76° C. indefinitely,[11] and in plasma frozen at −20° C. for 24 hours,[10] but not preservation in liquid whole blood for 72 hours[12] or in frozen plasma at −20° C. for 48 hours[10] or in plasma dried from the frozen state.[11]

Summary and Conclusions. 1. Findings on 35 administrations of plasma in dosages varying from 60 to 270 ml. prepared from patients

TABLE 1.—SUMMARY OF RESULTS OF ADMINISTRATIONS TO 35 PATIENTS OF PLASMA PREPARED FROM DONORS WITH ACTIVE MALARIA

Patient	Physical state in which plasma was preserved	Date blood drawn	Date plasma administered	No. of days of preservation	Dose in ml.	Type of malarial parasite	No. parasites/ oil immersion field on thick smear donor's blood	Result of injection	Remarks
1	Frozen	7/23/42	7/28/42	5	80	*P. malariæ*	3–4	Neg.	One attempt to infect pt. with whole malarial blood unsuccessful.
2	Frozen	7/23/42	7/28/42	5	80	*P. malariæ*	3–4	Neg.	Pt. came down with malaria 5 days after receiving 5 cc. malarial blood 13 wks. after plasma injection.
3	Frozen	7/23/42	7/28/42	5	80	*P. malariæ*	3–4	Neg.	
4	Frozen	7/28/42	8/ 4/42	7	160	*P. falciparum*	30–40	Neg.	Pt. came down with malaria 17 days after receiving 5 cc. malarial blood, 12 wks. after plasma injection.
5	Frozen	7/30/42	8/ 4/42	5	180	*P. falciparum*	15–20	Neg.	Pt. came down with malaria 19 days after receiving 5 cc. malarial blood, 12 wks. after plasma injection.
6	Frozen	8/ 7/42	8/19/42	12	80	*P. falciparum*	4–5	Neg.	
7	Frozen	8/ 7/42	8/19/42	12	80	*P. falciparum*	4–5	Neg.	
8	Frozen	8/ 7/42	8/19/42	12	80	*P. falciparum*	4–5	Neg.	
9	Frozen	9/ 2/42	9/ 9/42	7	150	*P. malariæ*	3–4	Neg.	
10	Frozen	9/ 2/42	9/ 9/42	7	100	*P. malariæ*	3–4	Neg.	
11	Dried	8/ 7/42	9/ 9/42	33	100	*P. falciparum*	4–5	Neg.	
12	Dried	8/ 7/42	9/ 9/42	33	100	*P. falciparum*	4–5	Neg.	
13	Dried	8/ 7/42	9/ 9/42	33	100	*P. falciparum*	4–5	Neg.	
14	Frozen	9/11/42	9/16/42	5	150	*P. malariæ*	1–3	Neg.	
15	Frozen	9/11/42	9/16/42	5	150	*P. malariæ*	1–3	Neg.	
16	Frozen	10/14/42	11/17/42	34	80	*P. malariæ*	10–15	Neg.	
17	Frozen	10/14/42	11/17/42	34	80	*P. malariæ*	10–15	Neg.	
18	Frozen	10/14/42	11/17/42	34	80	*P. malariæ*	10–15	Neg.	
19	Frozen	10/14/42	11/17/42	34	270	*P. malariæ*	10–15	Neg.	
20	Frozen	10/14/42	11/19/42	36	80	*P. malariæ*	10–15	Neg.	
21	Frozen	10/22/42	11/19/42	28	80	*P. malariæ*	4–5	Neg.	

13	Dried	9/11/42	9/16/42	5	150	*P. malariæ*	1–3	Neg.	
14	Frozen	9/11/42	9/16/42	5	150	*P. malariæ*	1–3	Neg.	
15	Frozen	10/14/42	11/17/42	34	80	*P. malariæ*	10–15	Neg.	
16	Frozen	10/14/42	11/17/42	34	80	*P. malariæ*	10–15	Neg.	
17	Frozen	10/14/42	11/17/42	34	80	*P. malariæ*	10–15	Neg.	
18	Frozen	10/14/42	11/17/42	34	270	*P. malariæ*	10–15	Neg.	
19	Frozen	10/14/42	11/17/42	34	270	*P. malariæ*	10–15	Neg.	
20	Frozen	10/14/42	11/19/42	36	80	*P. malariæ*	10–15	Neg.	
21	Frozen	10/22/42	11/19/42	28	80	*P. malariæ*	4–5	Neg.	
22	Frozen	10/22/42	11/19/42	28	80	*P. malariæ*	4–5	Neg.	
23	Frozen	10/22/42	11/19/42	28	80	*P. malariæ*	4–5	Neg.	
24	Liquid	11/20/42	11/21/42	1	100	*P. malariæ*	3–4	Pos.!	30 days after injection pt. showed clinical manifestations of malaria and 5–6/parasites/o.i.f. Required quinine.
25	Liquid	11/20/42	11/21/42	1	60	*P. malariæ*	3–4	Pos.?	28 days after injection pt. showed headache, fever to 100.4° F., no chills and 0–1/parasites/o.i.f. on 5 occasions. Required no treatment. Same donor as No. 24. Pt. had therapeutic quartan malaria in 1938.
26	Liquid	1/14/43	1/21/43	7	100	*P. malariæ*	6–7	Neg.?	8 wks. after injection pt. showed 0–1/parasites/o.i.f. on one occasion. No symptoms. Required no treatment. Pt. had therapeutic quartan malaria in 1933.
27	Liquid	1/14/43	1/21/43	7	100	*P. malariæ*	3–4	Neg.	
28	Liquid	1/14/43	1/21/43	7	100	*P. malariæ*	4–5	Neg.	
29	Liquid	1/14/43	1/21/43	7	100	*P. malariæ*	2–3	Neg.	
30	Liquid	1/14/43	1/21/43	7	100	*P. malariæ*	1–3	Neg.	
31	Liquid	1/14/43	1/28/43	14	100	*P. malariæ*	3–4	Neg.	
32	Liquid	1/14/43	1/28/43	14	100	*P. malariæ*	1–3	Neg.	
33	Liquid	1/14/43	1/28/43	14	100	*P. malariæ*	2–3	Neg.	
34	Liquid	1/14/43	1/28/43	14	100	*P. malariæ*	4–5	Neg.	
35	Liquid	1/14/43	1/28/43	14	100	*P. malariæ*	6–7	Neg.	Same plasma as No. 26 one week older.

with active malaria and preserved by varying techniques for varying periods of time are reported.

2. In 20 administrations of thawed plasma which had been "shell" frozen in a solid carbon dioxide-alcohol bath, no transmissions of malaria were observed.

3. In 3 administrations of restored plasma which had been dried from the frozen state, no transmissions were observed.

4. In 2 administrations of plasma preserved in the liquid state for 1 day, there was 1 definite transmission and 1 probable transmission. In 5 administrations of plasma preserved in the liquid state for 1 week, there was 1 very doubtful transmission. In 5 administrations of plasma preserved in the liquid state for 2 weeks no transmissions were observed.

5. It may be concluded that the likelihood of transmission of malaria by any plasma program, regardless of type of preservation used, is practically non-existent.

The suggestions of Captain E. G. Hakansson (M.C.), U. S. Navy, and Captain Paul W. Wilson (M.C.), U. S. Navy, and the technical assistance of the Blood Plasma and Parasitology Departments of the U. S. Naval Medical School, National Naval Medical Center, Bethesda, Md., are gratefully acknowledged.

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THE ACTION OF SPECIFIC STIMULATORS ON THE HEMATOPOIETIC SYSTEM

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(From the Charlotte Drake Cardeza Foundation, Jefferson Medical College)

The mechanism of the proliferation and maturation of blood cells is obscure, although assumptions based on the morphology of the blood and blood-forming organs have been made, and something is

known of the action of the anti-anemia factor of liver. Various factors that have been assumed to stimulate and inhibit the growth of blood cells have not been well demonstrated. K. Ziegler,[9] and later Wiseman, Doan, and Erf[8] postulated that a balance was maintained between myeloid and lymphoid tissues. They believed that hyperplasia of the one brought about hypoplasia of the other, but they were unable to explain the mechanism. In previous reports[3,4,5,7] it has been shown that proliferation of lymphoid and myeloid cells can be induced experimentally in guinea-pigs by the injection of specific materials derived from the urines of patients suffering from lymphoid or myeloid leukemia. This work has been confirmed recently by Heinle, and his co-workers.[1] Their results lack uniformity, and this may be ascribed to the lower potency of their material resulting from a less efficient extraction and purification method. The chemical fractionation of our specific stimulating materials has been described elsewhere.[6]

This communication describes the lesions induced in the organs of animals by the administration of our partially purified extracts and fractions of extracts obtained from the urines of patients with acute and chronic lymphoid leukemia, acute and chronic myeloid leukemia, monocytic leukemia, lymphosarcoma, and Hodgkin's disease. Two purposes have been realized, the accumulation of a large series of positive animals and the chemical concentration of the extracts of the active principles; the latter was accomplished by using the biologic test as a criterion of the chemical separation. The specificity of the biologic reaction, which has been emphasized previously, was of great value in the chemical work; it has permitted the collective description of the lesions produced in animals by various fractions that produced the same biologic reactions although obtained at different stages of the chemical concentration.

A theory is proposed concerning the action of the stimulating substances in inducing lesions in animals and the relation of these substances to the formation of similar lesions in some of the human blood dyscrasias.

Materials and Methods. Extracts of urine prepared by a method described in 1940[3] and fractions of these extracts described recently[4,6] were used in the present study.

Guinea-pigs, rats and rabbits have been used. The results have been consistently better in guinea-pigs; however, some of the most marked lymphoid changes have occurred in rabbits and rats. Since spontaneous leukemia is exceedingly rare in the guinea-pig, this animal was chosen for the investigation reported in this paper.

The original chloroform extract, which contained acid and neutral material, was adjusted to pH 7 to 8 with aqueous alkali. Each cubic centimeter of the aqueous solution containing suspended matter corresponded to 1000 to 1500 cc. of urine. It was injected in increasing doses, starting with $\frac{1}{4}$ cc.; the dose was increased every 5 days until $1\frac{1}{2}$ or 2 cc. was given daily. The first fractions obtained by the chemical separation of this extract were given either undiluted or in olive or sesame oil in 3 to 4 doses of 50 to 400 mg. per dose which represented similar or slightly greater amounts of urine. Subsequent fractions usually have been given undiluted. The doses of these fractions have been adjusted to permit a comparison of the two fractions produced by each procedure in the separation. Thus, when two fractions were obtained with 10

times as much material in one as in the other, these proportions were maintained in the amounts given of each part.

Blood counts of the animals were not of special significance. Most of the myeloid animals had some anemia and leukocyte counts of 40,000 to 50,000 were obtained several times. The hemograms of the lymphoid and myeloid animals were usually different. In the former, although the total leukocyte counts were normal, relative lymphocytoses occurred. In the latter, myelocytes, myeloblasts, and nucleated erythrocytes frequently appeared in the peripheral blood. The animals were killed as soon as they became moribund. This usually occurred 3 to 5 weeks after the beginning of the experiment. Most of the animals at necropsy were found to have necrosis and hemorrhage at the sites of injection. This occurred in negative as well as in positive animals.

Description of the Induced Lesions.* Thirty-six animals have been given the active principle, either as extracts or as fractions of extracts from the urine of patients with *chronic myeloid leukemia*. All these animals have exhibited changes in their organs similar to the changes of human chronic myeloid leukemia. Liver, spleens, adrenals, and bone marrows have shown myeloid proliferation invariably; the lungs, kidneys, and lymph nodes have been affected less frequently. In the liver, metaplasias of young erythroid and leukoplastic cells were found between the liver cords, around blood-vessels, and to some extent in periportal areas. Megakaryocytes occurred frequently in this new growth of young blood cells. The architecture of the spleen was disarranged because of the presence of myeloid proliferation, and a lack of lymphopoiesis was evident. In the adrenal, the metaplastic myelopoiesis has occurred under the capsule and in the cortex; less frequently, it has been noted in the medulla. In the cortex it has occurred as nests of forming cells between the cords or intravascularly. The bone marrows of all these animals have been hyperplastic in all the cellular elements (Figs. 1, 2).

Three animals have received the extracts of the urine from 2 patients with *acute myeloid leukemia*. The changes in the organs of these animals were exactly similar to those in the animals that received material from the urine of patients with chronic myeloid leukemia. The acute nature of the disease was not transferred (Fig. 3).

Forty animals received the lymphoid stimulating substance either as extracts or as fractions of extracts of the urine from patients with *chronic lymphoid leukemia*. These exhibited changes similar to those found in human chronic lymphoid leukemia. There was much less liver involvement than in animals that received myeloid substance, and this occurred as perivascular infiltrations or as minute lymph nodes. The spleens were always hyperplastic in cellular contents, often showing merely enlargement or increased numbers of lymph follicles; at times, however, the hyperplasia obliterated the entire splenic architecture. All the lymph nodes were somewhat enlarged and rupture of the capsule was noted several times. The kidneys were more often involved than in our myeloid animals. In animals that received the

* The accompanying photomicrographs represent sections of the organs of guinea-pigs, and illustrate the lesions produced by the active material obtained from various urines. The urinary source is indicated briefly following the identification of the organ. All sections were stained with hematoxylin and eosin.

lymphoid stimulating substance, the adrenals were less frequently involved than in animals that received myeloid stimulating substance, and when involvement occurred it manifested itself in the medulla rather than under the capsule or in the cortex (Figs. 4, 5).

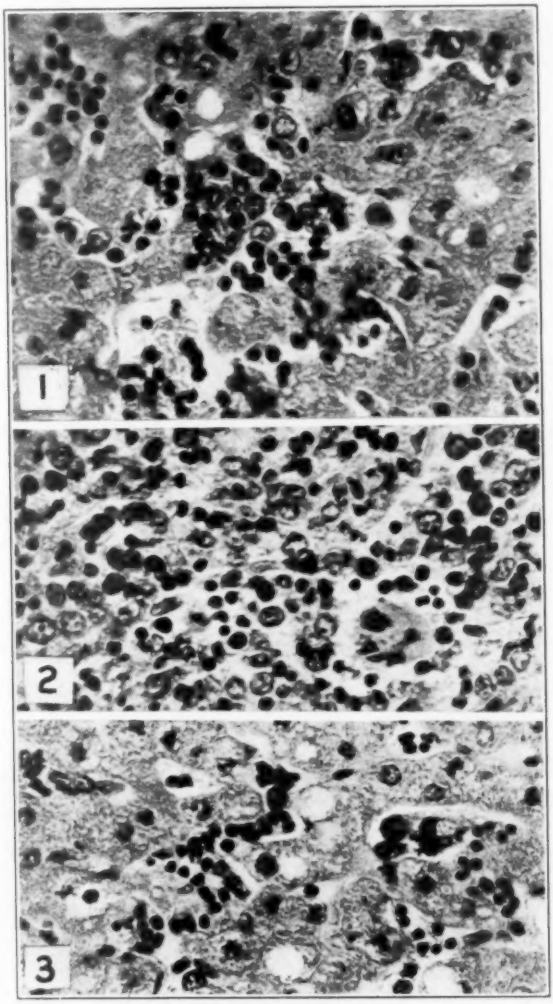
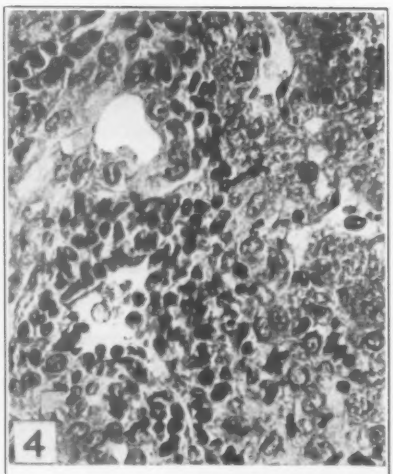
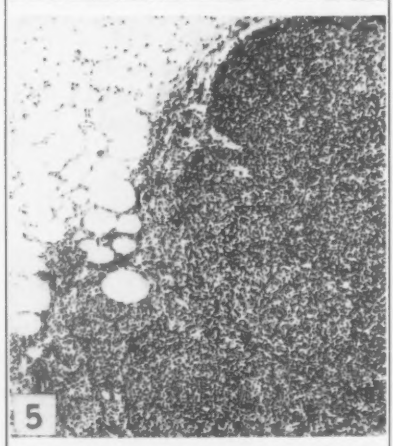
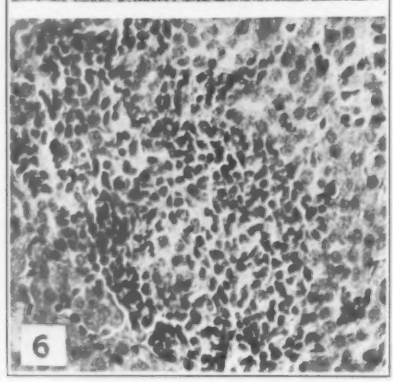


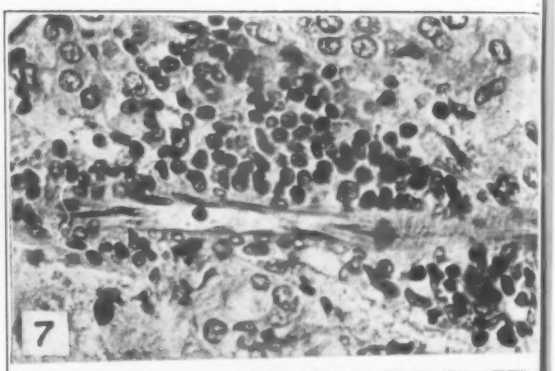
FIG. 1.—Liver: chronic myeloid leukemia. (× 475.)
FIG. 2.—Spleen: chronic myeloid leukemia. (× 475.)
FIG. 3.—Liver: acute myeloid leukemia. (× 400.)

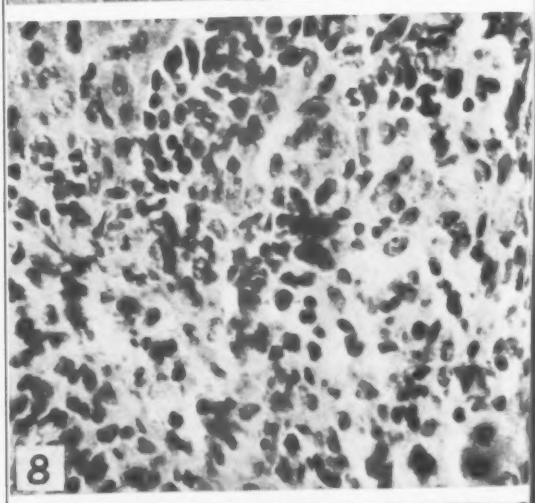
These same histologic changes were obtained in 8 animals given the active principle from the urine of 4 patients with acute lymphoid leukemia, although the lymphoid hyperplasia and metaplasia in non-lymphoid organs were somewhat greater than that elicited by chronic lymphoid extracts (Figs. 6, 7).











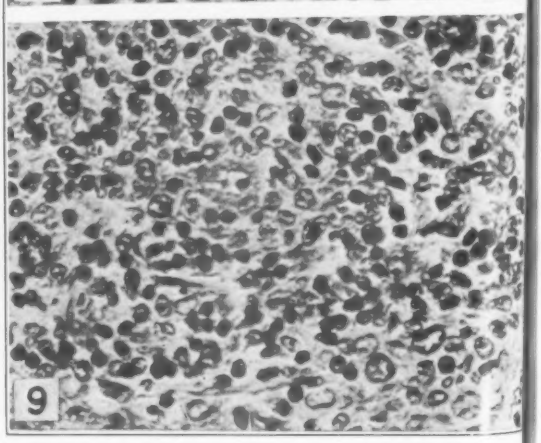


FIG. 4.—Liver: chronic lymphoid leukemia. (× 475.)
FIG. 5.—Lymph node: chronic lymphoid leukemia. Note the extension of lymphopoiesis through the capsule. (× 100.)
FIG. 6.—Liver: acute lymphoid leukemia. (× 400.)
FIG. 7.—Adrenal: acute lymphoid leukemia, hydroxy-acid fraction. (× 475.)
FIG. 8.—Liver: Hodgkin's disease. (× 475.)
FIG. 9.—Spleen: Hodgkin's disease. (× 475.)

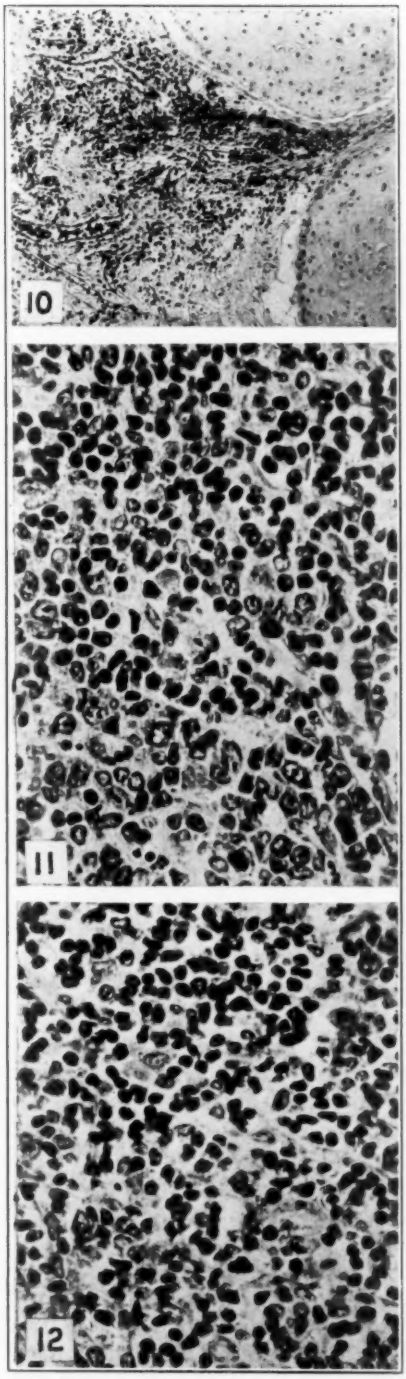


FIG. 10.—Trachea: Hodgkin's disease. Note the infiltration in tissue between cartilage. (× 100.)

FIG. 11.—Spleen: Hodgkin's disease, hydroxy-acid fraction, showing lymphoid hyperplasia. (× 475.)

FIG. 12.—Lymph node: Hodgkin's disease, hydroxy-acid fraction, showing lymphoid hyperplasia. (× 475.)

Again, to a lesser degree, lymphoid hyperplasia and pictures simulating lymphoid leukemia were obtained in 8 animals after the use of the partially purified extracts of urine from 4 patients with *lymphosarcoma*.

Four animals were given the partially purified extract of the urine from 2 patients with *monocytic leukemia*. The changes elicited in the organs of these animals were neither lymphoid nor myeloid. The liver showed some perivascular infiltration with small round cells similar to lymphocytes, and at times this infiltration contained many eosinophils. The liver of 1 animal showed minute nodules composed of round cells, eosinophils, and fibroblasts. The spleens contained many large cells with foamy cytoplasm, many fibroblasts, and a few multinucleated giant cells. The lymph nodes were somewhat enlarged in all these animals and the histologic changes were similar to the changes in the spleens. All the bone marrows were hyperplastic, and there was always

FIG. 13.—Lymph nodes surrounding trachea: chronic myeloid leukemia, hydroxy-acid fraction, showing lymphoid hypertrophy. (× 8.)

an increased number of megakaryocytes. In all these lesions the number of mitotic figures also was increased.

The same changes occurred in the organs of 6 animals treated with similar material from 2 patients with Hodgkin's disease. In these, however, a more definite trend towards lymphoid stimulation was apparent. In 2 animals, the lymph nodes had the appearance of lymphosarcoma with proliferation of cells through the capsules and, in one instance, the nodes found near the trachea showed infiltration between the cartilages of this organ. Again, there was an increased number of mitotic figures in the lesions (Figs. 8, 9, 10).

Each of 3 animals was given equal weights of lymphoid and myeloid stimulating fractions simultaneously. These fractions were both obtained at a similar stage of the fractionation procedure described elsewhere[6] and corresponded to acids B of that paper. The changes in the histology of the organs of these animals resembled the changes

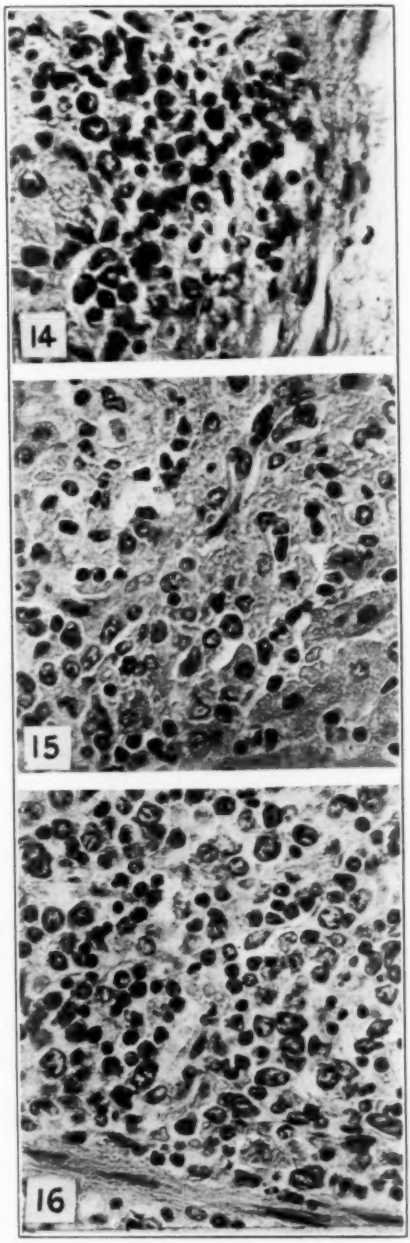


FIG. 14.—Adrenal: chronic lymphoid leukemia, hydroxy-acid fraction oxidized and non-carbinol acids separated from the product and injected. This shows a myeloid reaction beneath and in the capsule. (× 475.)

FIG. 15.—Liver: chronic lymphoid leukemia, non-carbinol acid fraction. Note the myeloid reaction between liver cords. (× 475.)

FIG. 16.—Spleen: chronic lymphoid leukemia, non-carbinol acid fraction. Note the erythropoiesis and myelopoiesis. (× 475.)

found in the animals that received extracts of the urine of patients with Hodgkin's disease or monocytic leukemia.

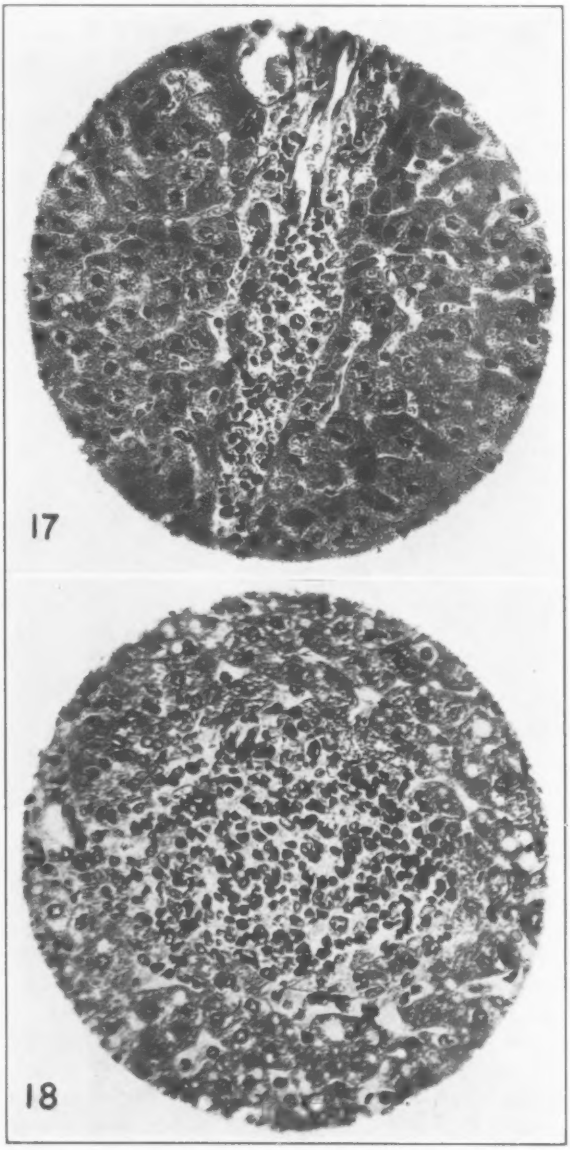


FIG. 17.—Liver: injection of equal weights of myeloid and lymphoid stimulating fractions. This shows perivascular lymphoid infiltration accompanied by eosinophils. (× 475.)

FIG. 18.—Liver: monocytic leukemia, non-carbinol acid fraction reduced and hydroxy-acids separated from the product and injected. This shows a lymphoid reaction. (× 475.)

In our paper describing the chemical aspects of this problem[6] it has been shown that mixtures of the myeloid stimulating substance and the lymphoid stimulating substance can be separated. The lymphoid stimulating material was removed from the mixture in the fraction containing hydroxy-acids, while the myeloid stimulating material remained with the non-carbinol acids. By employing this procedure it was possible to show the presence of small amounts of lymphoid stimulating material together with large amounts of myeloid stimulating material in the urine of patients with chronic myeloid leukemia.

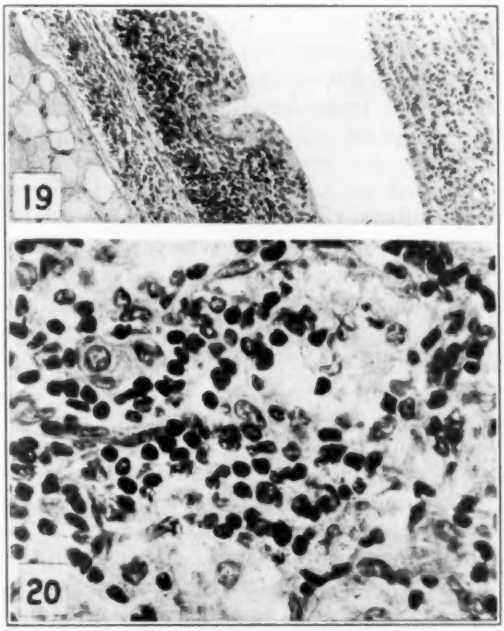


FIG. 19.—Pelvis of kidney: same material as Figure 18. (× 100.)
FIG. 20.—Medulla of adrenal: same material as Figure 18. (× 475.)

The urine of patients with monocytic leukemia or Hodgkin's disease was found to contain the two specific myeloid or lymphoid stimulating substances in a more equal proportion. The mixtures were separated in the same manner and the hydroxy-acid fraction gave a pure lymphoid response in animals while the non-carbinol acids gave a pure myeloid picture in animals.

The urine of patients with chronic lymphoid leukemia was shown to contain small amounts of myeloid stimulating material besides large amounts of lymphoid stimulating material.

No myeloid stimulating substance was found in the urine of 2 patients with acute lymphoid leukemia.

The biologic results supporting these chemical separations are shown

in Chart 1. The degree of activity of each fraction is also noted in Chart 1.

CHART 1.—ACTIVITY OF CHEMICAL FRACTIONS

Material	Myeloid leukemia		Lymphoid leukemia		Monocytic leukemia	Hodgkin's disease
	Chronic	Acute*	Chronic	Acute		
Hydroxy-acids:						
Lymphoid activity	+ (2)	— (2)	+++ (2)	+++ (2)	++ (2)	+++ (2)
Non-carbinol acids:						
Myeloid activity	+++ (2)	++ (2)	+ (2)	— (2)	+++ (2)	++ (4)

* Since the manuscript was sent to press, one urine of a patient with acute myeloid leukemia has become available and the result entered on this chart.

The number of animals employed is indicated in parentheses.

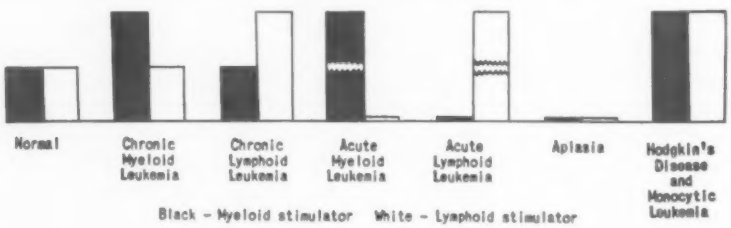
In the same report,[6] a chemical method was described for the conversion of the lymphoid stimulating substance to myeloid stimulating material. The reverse transformation was accomplished also. This involved a reduction for the conversion of myeloid material to lymphoid and an oxidation for the reverse change. These reactions in each instance were applied to material containing only one of the two biologically active substances (Figs. 11 to 20).

Discussion. The chemical evidence reported elsewhere[6] suggests strongly that there are only 2 substances involved in producing the specific histologic changes described in this paper, although a variety of urinary sources has been used. These substances, one of which produces myeloid proliferation, and the other lymphoid, are closely related chemically.

In order to explain the action of the 2 substances we have formulated a theory which accounts in part for their physiologic activity.

In the amounts we have used, no active material has been found in the urine from normal individuals. We assume, however, that the body fluids of normal individuals must contain similar substances and that normal hematopoiesis is regulated by them. On this assumption and from our findings we have charted in a rough way the occurrence of these substances in the normal and in several of the blood dyscrasias (Chart 2).

CHART 2.—THEORETICAL OCCURRENCE OF STIMULATING SUBSTANCES IN THE NORMAL AND SEVERAL OF THE BLOOD DYSCRASIAS



We suggest that these substances are mutually reciprocal in action. The myeloid substance stimulates myelopoiesis, *i. e.*, proliferation

without maturation. The maturation of myeloid cells is brought about by the action of the lymphoid substance which inhibits the proliferation of the myeloid cells and hence allows them to mature. The lymphoid substance brings about lymphoid proliferation without maturation. Maturation of these cells is brought about by the action of the myeloid substance which inhibits the proliferation of the lymphoid cells and hence allows them to mature. Normally, the 2 substances are balanced in action and therefore regulated hematopoiesis occurs. We postulate that an excess of myeloid substance occurs in chronic myeloid leukemia together with at least a normal amount of lymphoid substance. Hence the disease runs its course with greater than normal maturation of myeloid cells in evidence. As the lymphoid substance becomes exhausted, an acute exacerbation occurs, characterized by proliferation of myeloid cells without maturation. Death follows with the disease in an acute or blastic phase. Chronic lymphoid leukemia may be explained in a similar manner.

The two acute diseases, acute lymphoid and acute myeloid leukemia, may be explained as deficiencies of one or the other maturating substance.

By giving lymphoid or myeloid substance to animals we have experimentally reproduced the two diseases in which an excess of one substance and at least a normal amount of the other are assumed to occur. The detection of both substances in the urines of patients with these two diseases substantiates this assumption, and also explains the inability to reproduce the acute phases of these diseases. If one or the other substance could be destroyed or removed from the animals the corresponding deficiency disease easily would be obtained. In the extracts of urine of 2 patients with acute lymphoid leukemia we were unable to demonstrate myeloid substance. Theoretically, we should not obtain more than one substance from the urines of patients in the acute phase of leukemia.

Hypoplastic or aplastic anemia may be assumed to be a deficiency disease in which both factors are lacking; therefore, little proliferation and little maturation of either type of cell takes place.

We have found both stimulating substances in excess in the urine from patients with Hodgkin's disease and monocytic leukemia; therefore we have placed these two diseases in that category on Chart 2. The reaction observed in the animals that received both substances substantiates this part of the hypothesis. Lymphosarcoma is omitted from the chart, but from this work it would seem to be a variation of lymphoid leukemia.

This work may lead to a new type of therapy for human leukemia.[2] If the theory is correct, leukemia resulting from a lack of one of the 2 substances may be controlled by the replacement of this substance. The theory, however, implies that such replacement therapy would be useful in the chronic leukemias only in preventing the occurrence of acute or blastic phases.

Summary. Descriptions and photomicrographs are given of lesions observed in animals following injection of 2 substances obtained from

urines of patients with leukemia, and allied diseases. A theory is proposed to account for the action of these substances. This work may provide a basis for a better understanding of leukemia and of normal hematopoiesis.

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SICKLING TRAIT IN A WHITE ADULT ASSOCIATED WITH HEMOLYTIC ANEMIA, ENDOCARDITIS AND MALIGNANCY*

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Until Cooley and Lee[3] first reported an authenic case of sickle-cell anemia in a white person, the disease was assumed to be characteristically one of the negro race. Additional trustworthy cases[1–6,8,8a,10–14,17] have subsequently accumulated; the pertinent data of the known cases are summarized in Table 1. It seems more than a coincidence that these, and the patient to be reported, are all of South Italian stock. The not infrequent occurrence of the sickle-cell trait in Puerto Ricans and Mexicans is understandable, because of the high incidence of cross-breeding with the negro race. In tracing the derivation of those patients whose family history admits of no such admixture, one is struck by the high proportion with Mediterranean background.

It is a moot question, whether, as some students of the disease predicate, a negro strain was introduced during the migrations and wars of ancient or even earlier times, or whether the sickle-cell trait need not

* Aided by a grant from John Wyeth & Brother, Inc.
† Dr. Spielholz is now on active service.

carry any connotation of negro heritage. The subject has more than ethnologic or historic interest, for it may be a clue to the diagnosis and understanding of a hitherto bizarre and unrecognized group of hemolytic anemias. For this reason, it is suggested that the blood in all cases of obscure hemolytic anemia be tested for the sickling trait.

An additional case of hemolytic anemia in a white person who showed the sickling trait and in whom a metastatic carcinoma was found on postmortem examination, is presented in this communication.

TABLE 1.—RÉSUMÉ OF PREVIOUS REPORTED CASES OF SICKLE-CELL ANEMIA IN WHITE PERSONS

Author	Year	Age	Sex	Birthplace	Father's birthplace	Father's blood	Mother's birthplace	Mother's blood	Remarks
Cooley and Lee[3]	1929 1929	4 yrs. 14 yrs.	M M	U. S. U. S.	Byregos, Greece	Normal	Byregos, Greece	Sickles	2 siblings, normal
Sights and Simon[12]	1931	48 yrs.	M	U. S.	U. S.	Not exam.	U. S.	Not exam.	Patient had lues and malaria, transient sickling; Scotch-Irish descent
Rosenfeld and Pincus[11]	1932	9 yrs.	M	U. S.	Reggio, Calabria, So. Italy	Normal	U. S., parents from Casabana, Calabria	Sickles, long-standing anemia	Maternal grandmother and aunt and 1 sibling sickle
Clarke[1]	1933	3 yrs. 11 yrs.	M M	U. S. U. S.	Carlentina, Sicily	Normal	Carlentina	Normal	3 siblings, normal
Cooke and Mack[2]	1934	10 mos. 3½ yrs.	M F	U. S.	U. S. (Ohio)	Sickles	U. S., parents from Ky., Va.	Normal	4 siblings have palpable spleen, no sickling
Haden and Evans[5]	1937	8 yrs. 15 yrs.	F F	Sicily U. S.	Sicily	Died of blood dyscrasia atypical anemia	Sicily	Normal	1 brother sickles, 4 siblings normal
Johnson and Townsend[6]	1937	5 yrs.	M	Greek	Greece	Dead	Greece	Normal	3 siblings not examined
Weiner[17]	1937	8 yrs.	M M	U. S. N. J.	Mistressa, Sicily	Normal	Mistressa, Sicily	Normal	Maternal grandmother neg., 1 sister neg., 2 younger brothers show anemia, sickling and splenomeg.
Pontoni[10]	1939	13 yrs.	F	Italy					Quotes 2 cases previously reported in the Italian literature
Greenwald and Burrett[4]	1940	22 yrs. 9 yrs.	F F	U. S. U. S.	Naro, Sicily	Not exam. dead	Naro, Sicily	Sickles	Mother, aunt and cousin sickle
Mallory[8,8a]	1941	20 yrs.	F	U. S. Boston	Italy	Refused exam.	Italy	Normal	2 siblings negative
Wade and Stevenson[14]	1941	49 yrs.	F	Greek					No familial history
Vance and Fisher[13]	1941		M		Greek parentage				No additional family history

Case Study. L. D., admitted on Aug. 13, 1941. Female, white, age 54; born in Palmerno, Italy.

Present History. Weakness, fever, generalized aches and pains and headache were first noted on August 3, and became progressively worse. A "black-eye" and several other areas of ecchymoses were noted 2 days before admission.

Family and Past History. The patient was an adopted child and nothing is known of her parents, though they came from the same village as her foster parents, where negroes were unknown. She is said to have had no siblings. She had enjoyed reasonably good health. Para vii; 6 children are alive and well. One daughter died of hemorrhage following childbirth. Menopause was uneventful two years ago. The patient was operated upon in March 1941, when a cholecystectomy was performed because of a phlegmonous gall bladder.

Physical Examination.—On admission the patient was extremely ill, temperature 101.6° F., pulse 80 to 120, respiration 20 to 30. She was pale and jaundiced, obese, and there were no negroid features. There was a periorbital ecchymosis of the right eye, and a few scattered petechiæ and ecchymoses. Tachycardia was noted, and there was a blowing systolic murmur heard best over the pulmonic area; blood pressure 112/55. The lungs were clear. The abdomen was soft and not tender. The liver and spleen were not felt. A transverse scar was noted in the right upper quadrant. There was no peripheral edema, and reflexes were normal.

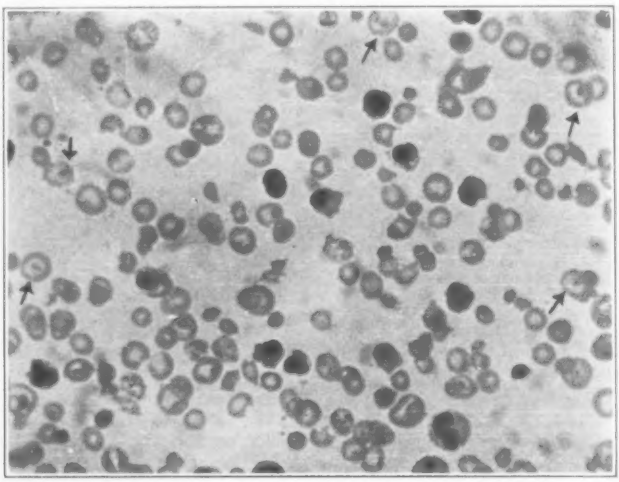

FIG. 1.—Peripheral blood smear showing erythroblastosis and target cells (some are marked by arrows). (× 1350.)

Laboratory Data. Peripheral blood count the day following admission: Hgb. 25%, R.B.C. 1.30 million, W.B.C. 26,000; differential: myeloblasts 2%, neutrophilic myelocytes 3%, metamyelocytes 6%, staff forms 30%, segments 25%, eosinophils, 3%, lymphocytes 30%, monocytes 1%, normoblasts 55 and erythroblasts 5 per 100 W.B.C. (Fig. 1), reticulocytes 17.6%, R.B.C. showed slight anisocytosis, hyperchromia and polychromasia. There was slight poikilocytosis. The bone marrow (aspiration) was hyperplastic and erythroblastic, with good distribution of the myeloid elements. Few megakaryocytes were seen (Fig. 2). Platelet count: 60,000 platelets, which dropped in 5 days to 15,000. Subsequent blood counts are recorded in Table 2. Coagulation time was $3\frac{1}{4}$ minutes; bleeding time, $2\frac{1}{4}$ minutes. Clot retraction began in 1 hour and was complete in 15 hours. The tourniquet test was positive. Hemolysis to hypotonic saline began at 0.495 and was complete at 0.270. Icteric index was 28 on admission and rose to 80, with a positive immediate

direct Vandenburg reaction. Sickling of the R.B.C. in a "wet-slip preparation" was demonstrated on several occasions (Fig. 3). Blood chemical figures per 100 cc. of blood were: N.P.N. 25 mg., glucose 130 mg., total cholesterol 180 mg., cholesterol esters 68 mg., creatinine 1.5 mg., total protein 7.83 gm.; albumin, 4.64 gm., globulin 2.89 gm., fibrinogen 0.3 gm., prothrombin index 78%; gastric analysis free acid absent after histamine. The urine showed no hemoglobinuria. Roentgen ray of chest (Aug. 14, 1941) "the transverse diameter of the heart shadow is somewhat increased. The hilar and parenchymal marking are increased. Impression: cardiovascular disease." E.C.G. (Aug. 14, 1941): "Q_1 absent, T_1 low. Left axis deviation. Impression: possible myocardial damage."

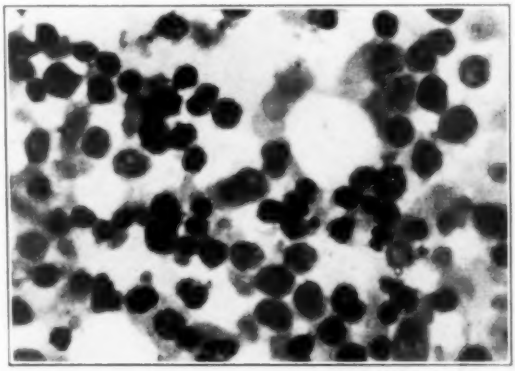

FIG. 2.—Sternal marrow smears showing hyperplastic erythro-normoblastic marrow. (× 1350.)

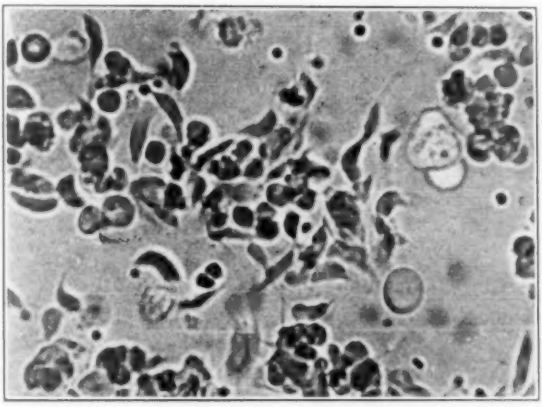

FIG. 3.—Wet preparation showing marked sickling of erythrocytes. (× 1350.)

Course. Frequently repeated blood transfusions (9 in all), large doses of cevitamic acid and parenteral liver extract had little effect upon the clinical course or upon the blood picture. The patient died on August 30, 1941.

Necropsy. GROSS (Dr. Andrea Saccone, of Metropolitan Hospital): *Heart.* 450 gm. The apex is covered with a slight amount of yellow fat. The right ventricular wall measures 5 mm. in thickness, the left, 1 cm. On the corpora Arantii there are some very fine vegetations. The same type of vegetations

is present on the mitral valve. There is no change in the tricuspid valve. The foramen ovale is closed. The myocardium is uniformly dull in appearance, but there are some grayish areas in the myocardium supplied by the anterior descending coronary artery. The circumflex (left) coronary artery on section shows only some patches of sclerosis. The aorta has a reddish-yellow intima and, starting at the arch, there are some patches which are raised.

Respiratory Apparatus. The larynx, trachea and large bronchi are covered with a slight amount of yellowish mucus. The glands at the hilus of the lung are small, discrete and dark in color. The left lung weighs 450 gm. It is slightly yellowish in color with small areas of atelectasis. There is a small area of atelectasis, with its base, measuring 1 cm., at the lower edge of the lung. Large amounts of foamy fluid can be expressed from the lung. The right lung weighs 550 gm. The lower lobe is dark red in color and firm. On section it has the same appearance as the left lung.

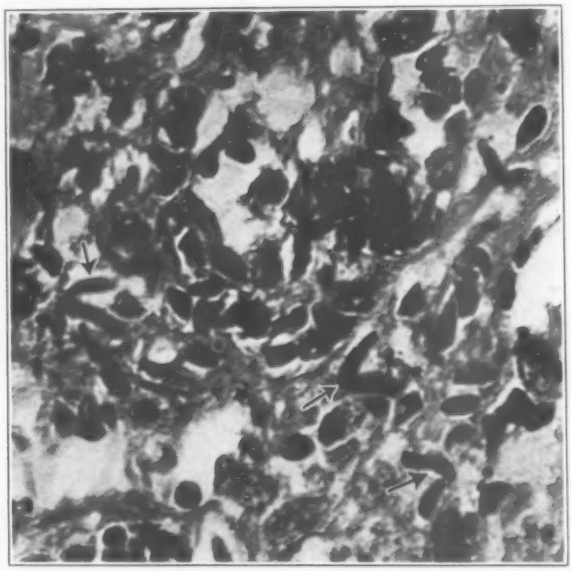

FIG. 4.—Spleen showing sickle-cells (arrows) in pulp spaces near a trabecula. (× 1125.)

Spleen: 180 gm. The capsule is tense and at the edge of the capsule there are some dark areas which are sharply demarcated on section. The pulp is firm with prominent trabeculæ.

Pancreas. 180 gm. There is an increase of the connective tissue on section.

HISTOLOGIC (Dr. F. D. Speer): *Spleen* (Fig. 4) shows moderate congestion. The capsule and trabeculæ show a slight increase in fibrillar connective tissue. Small and moderate sized collections of lymphoid tissue are noted about the splenic arterioles. No active "germinal centers" are seen. The arterioles themselves show slight thickening of their walls due to an increase of fine fibrillar connective tissue. High-power study of the splenic pulp shows a moderate number of elongated and sickle-shaped red blood cells throughout. It is estimated that this cell type comprises about 10% to 15% of the red cells seen in the pulp. There is no apparent increase in leukocytes or in splenocytes in the pulp. Another section of the spleen shows a small triangular-shaped subcapsular area in which the finer pulp structures shows slight granular disintegration. Immediately beneath the capsule there is a narrow zone of congestion

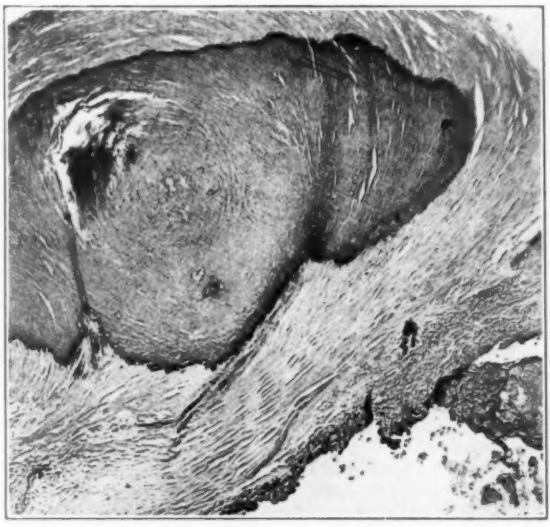

FIG. 5.—Part of mitral valve, showing free edge with large calcific and fibrotic area, endothelial surface destroyed over one portion with laminated fibrinous thrombus. (× 175.)

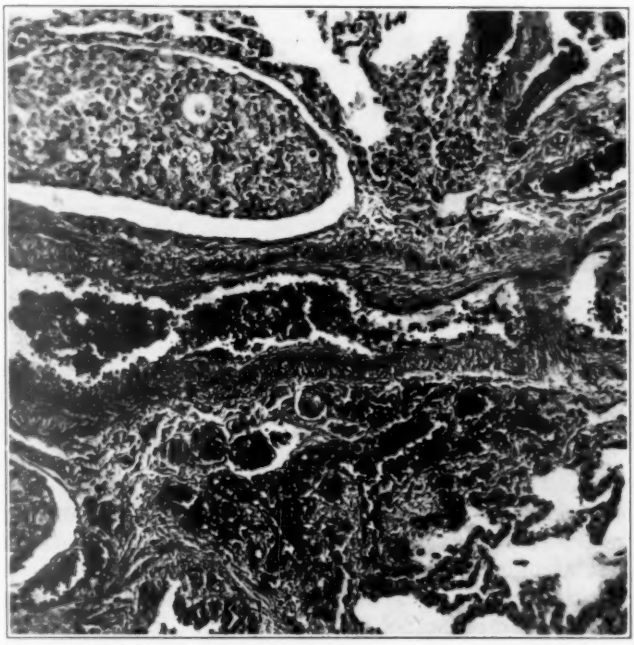

FIG. 6.—Lung showing small artery and bronchiole and carcinomatous metastasis in adjacent tissue. (× 1125.)

and hemorrhage. Throughout this zone, and also throughout the pulp tissue, there is a number of small blood-vessels occluded by thrombi in which fibrin and a few red and white blood cells can be identified. There is also an extensive zone of congestion and hemorrhage in the pulp and adjacent to the area of granular necrosis. In these areas of congestion and hemorrhage, small numbers of sickle-shaped red blood cells are seen.

Pathologic Diagnosis. Fibrosis, moderate congestion, moderate numbers of sickle-shaped red cells in pulp, small recent infarct showing adjacent hemorrhage, focal thrombosis of the smaller blood-vessels.

Mitral Valve (Fig. 5) shows a pyramidal segment of myocardial muscular tissue at the base. The valve leaflet shows a minimal increase in hyalinized connective tissue. At the edge of the valve there is a large knoblike thickening consisting of laminated hyalinized connective tissue and a calcified center. The surface of the valve covering this nodule shows an irregularly shaped thrombotic mass consisting essentially of old fibrin in which there are a few entrapped red blood cells and leukocytes. No bacterial colonies are demonstrated in this thrombotic mass. In this section of valve and attached myocardium there are no Aschoff lesions.

Pathologic Diagnosis. Focal fibrosis and calcification (healed rheumatic valvulitis). Small valvular thrombi consisting of old fibrin (thromboendocarditis).

Liver. Central albuminous and fatty degeneration and cytonecrosis, focal fatty infiltration, periportal fibrosis, focal fibrin thrombi in sinusoids, isolated sickle-shaped red blood cells.

Lungs (Fig. 6). Focal atelectasis and edema, multiple small carcinomatous metastases (bronchogenic origin?).

Peritracheal Lymph Nodes. Anthracosis, metastatic anaplastic carcinoma (bronchogenic origin?).

Pancreas. Slight peripancreatic fibrosis, minute carcinomatous metastases in peripancreatic tissues. Multiple carcinomatous metastases and fibrosis of lymph nodes.

TABLE 2.—BLOOD FINDINGS OF THE PATIENT

Date	Hgb. (%)	R.B.C.	W.B.C.	Platelets	Myeloblasts	Myelocytes	Metamyelocytes	Stab	Segmented	Lymphocytes	Monocytes	Eosinophils	Reticulocytes (%)	Nucleated R.B.C.	Morphology
8/14	25	1.30	26,000		2	3	6	30	25	30	1	3	17.6	60	Aniso. macro. microcytosis, polychromasia, hyperchromia, occasional sickle cells, target cells
8/18	25	1.25	36,300	60,000											"
8/20	30	1.45	24,100		1	2	8	16	40	30		3	21.0	157	"
8/23	25	1.53	30,300												"
8/25						3	11	16	37	28	3	2	19.2	95	"
8/26	22	1.18	30,800	15,000											"
8/28					1	5	11	14	26	36	2	5	20.0	133	"

Following the recognition of sickling of the erythrocytes in the patient all of her descendants were examined for the sickle-cell trait. Latent sicklemia was demonstrated in 4 members of the family: 2 sons, a daughter and a grandchild (see Chart 1). They are all well and give no history suggestive of previous hemolytic crises nor show any hematologic evidence at present, of active disease (Table 3). It will be noted that an increased fragility to hypotonic saline was found in 3 of these cases, as well as in the patient. This is contrary to the usual finding of increased resistance.

Discussion. There are several points of interest in the above story. The advanced age of the patient, the finding of malignancy and the rela-

tionship of this finding to the differential diagnosis of the anemia, the endocardial lesion demonstrated, and the associated thrombocytopenia are all unusual and deserving of some comment.

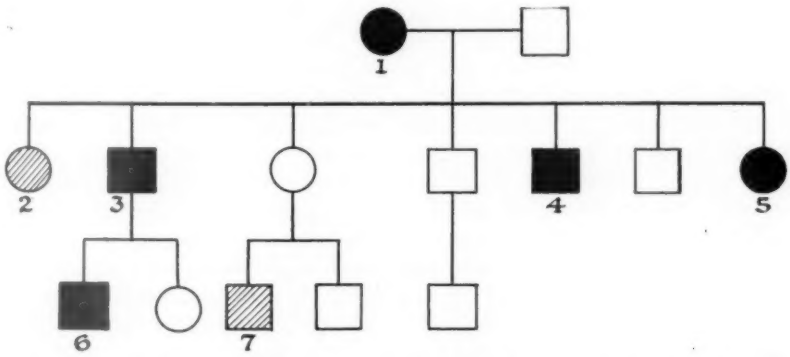

CHART 1.—Black circles (females), rectangles (males) indicate those who showed sickling of erythrocytes. (1) The patient; (3, 4, 5) her two sons and daughter; (6) a grandson; (2) daughter died of hemorrhage at childbirth; (7) grandchild died of pneumonia at the age of 1 year.

TABLE 3.—BLOOD FINDINGS IN THE DESCENDANTS SHOWING THE SICKLE-CELL TRAIT

Name	Sex	Age (yrs.)	Hgb. (%)	R.B.C.	W.B.C.	Platelets	Reticulocytes (%)	Stab	Segmented	Eosinophils	Lymphocytes	Monocytes	Fragility, R.B.C.	Icteric index	Wassermann	Morphology
F. D.	M	36	95	4 95	7,800	230,000	0 2	3	69	1	25	2				Normal red cells
L. D.	M	28	100	5 06	8,800	350,000	0 2	1	58		35	6	0 52 to 0 32	4	Neg.	Rare target cell
A. D.	F	23	81	4 40	6,200	340,000	0 2	3	57	3	30	7	0 52 to 0 32	6	Neg.	Rare target cell, rare sickle cell
F. D., Jr.	M	12	76	4 45	6,000	300,000	0 4		49	3	38	10	0 48 to 0 32		Neg.	Slight anisocytosis, rare target cell

This is the oldest in the group of non-negro patients collected in Table 1. With the exception of Wade and Stevenson's[14] 49 year old patient, and Sights and Simon's[12] 48 year old patient (which has been included but not definitely accepted), the patients were all in the first or second decades of life. Nothing in our patient's history suggested any hemolytic crisis before the fatal one at 54 years of age. Although sickle-cell anemia usually begins in childhood, the first symptoms of the disease have been observed in a negro 78 years old.[18] No iron deposition was found in the liver nor in the cells of the reticuloendothelial system. The moderate fibrosis of the spleen, as noted, is the only suggestion that earlier mild episodes of hemolysis may have taken place.

Clinically and hematologically the findings associated with a hemolytic crisis dominated the picture, *viz.*, anemia, icterus, reticulocytosis,

macrocytosis and normoblastosis. The thrombocytopenia and hemorrhagic diathesis are unusual, but have been reported.[9] The etiologic factor accounting for the acute hemolytic episode was obscure until illuminated by the demonstration of the sickling trait. Differential diagnosis suggested several possibilities. Cancer, ultimately found by microscopic examination of the organs, though considered, could not be demonstrated clinically, and the patient was too ill to be subjected to rigorous diagnostic procedures. Acute hemolytic anemia of the so-called "Lederer" type was a tentative diagnosis. Failure to respond to repeated transfusion tended to remove her from this group. The indication for splenectomy was considered in view of the lack of response to transfusions, but discarded because of the discovery of sickling and the critical condition of the patient. Bone marrow studies ruled out a possible leukemia, suggested by the leukemoid peripheral white cell response. A careful search into her dietary habits failed to confirm favism as a possibility. No history of drugs nor contact with cyclic chemicals could be obtained.

Before the necropsy report, the case obviously appeared to be one of sickle-cell anemia in crisis. The postmortem findings of metastatic carcinoma and an endocardial lesion offered two additional possible causes for the hemolytic crisis. Any of these conditions may have produced this type of anemia. The absence of bacterial growth on the endocardial lesion and the necropsy findings tended to rule out infection as the cause of the anemia. Unfortunately no blood culture was done. There are several observations which incline toward the view that hemolysis was secondary to sicklemia rather than to malignancy. *A.* Sickle cells were demonstrated in the spleen, liver and in the heart's blood postmortem. It is accepted that actual sickling of cells within the internal organs occurs only in the active phase of the disease, whereas in latent cases, *in vitro* lowering of oxygen tension is necessary, to produce sickling. *B.* The malignant lesions were small, subclinical, found only on histologic sections and metastasis were apparently limited to the lymph drainage areas in the lungs, peritracheal lymph nodes, peripancreatic tissue and lymph nodes. Repeated search of bone marrow material failed to disclose any cells suspicious of malignant characteristics. Though reported,[16] hemolytic anemia due to malignancy is admittedly quite rare. Waugh's[16] cases showed extensive metastasis to the bones, either by Roentgen ray or direct examination. *C.* The violently acute onset of the clinical course, its rapid downhill progress, and the domination of the picture by the hemolytic anemia syndrome seem to be more in favor of sickling as the etiology. *D.* And finally, the moderate amount of splenic fibrosis found suggests one or more previous unrecognized episodes of mild hemolysis. Fragility to hypotonic saline was somewhat increased. This is more concordant with Waugh's observations in 2 cases of malignancy with hemolytic anemia and with one similar case seen by the writers. Sickle-cell anemia, on the other hand, is usually associated with increased cell resistance. It should be noted, however, that increased fragility was found in 3 of the patient's descendants who showed sicklemia.

The endocardial lesions were described as focal fibrosis and calcification of the mitral valve (healed rheumatic valvulitis). No previous history suggestive of rheumatic fever had been obtained. Although the similarity between manifestations of acute crisis of sickle-cell anemia and rheumatic fever have been pointed out repeatedly, and the diagnosis of rheumatic fever has been made mistakenly many times, the literature contains only 1 case[15] of both diseases coëxisting. Klinefelter,[7] writing recently, but probably before Walker and Murphy's[15] case was published, gives an excellent résumé of the heart in sickle-cell anemia and stresses that "although the diagnosis of rheumatic heart disease has often been made in patients with sickle-cell anemia, it has not been confirmed at autopsy." In our case, the lesion, unsuspected during life, was a chance postmortem observation.

Though thrombocytopenia and hemorrhagic diathesis may occur in either sickle-cell anemia or metastatic carcinoma, its presence is noted again as unusual.

The possibility of sickle-cell anemia in all cases of obscure hemolytic anemia regardless of race should be borne in mind. While rarer in whites than in negroes, this disease may be more common than supposed, and may be overlooked because it is not customary to examine wet-sealed blood preparations routinely. It should be noted that, through the cases reported and summarized here, additional blood relatives, unsuspectingly bearing the trait, have been revealed. Any of these may, in the future, become active cases. Undoubtedly, many others showing sicklemia would be found if wide-range examinations of ethnic groups other than negroes were done, especially among Mediterranean peoples.

Parallelism between erythroblastic anemia of Cooley and sickle-cell anemia has been pointed out by many. Both conditions are basically congenital defects of the red corpuscle occurring predominantly in Mediterranean peoples, and manifesting themselves as hemolytic anemia frequently associated with similar bone changes.

Summary and Conclusions. 1. A case of sickle-cell anemia in a white person is reported and the literature summarized. The case is associated with metastatic carcinoma.

2. Familial occurrence of the sickle-cell trait in offspring of the patient has been demonstrated.

3. An associated rheumatic endocardial lesion was present; this has been reported only once before.

4. The advanced age (54 years) for a first and fatal attack of hemolytic crisis is noted and the relationship to the coëxisting malignancy discussed.

5. The relatively frequent occurrence of sickle-cell anemia among people of southern Italian and Mediterranean descent is again noted.

6. The need to consider the diagnosis of sickle-cell anemia in all cases of obscure hemolytic anemia is once more stressed.

We wish to thank Prof. Linn J. Boyd of the Department of Medicine for permission to report this case and for his valuable aid.

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MAINTENANCE OF THE SEDIMENTATION RATE AS A TEST FOR MALIGNANT DISEASE

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It is well known that the speed with which the erythrocytes in citrated or oxalated blood settle is retarded if the blood is allowed to stand for several hours before the test is set up. Koster[2] and Feldman[1] have reported the observation that this retardation is largely absent in the blood of patients with malignant tumors and Hodgkin's disease, so that the sedimentation rate after storage remains near the initial rate.

Koster[2] described 3 types of behavior in the blood from patients with cancer:

"Type I. The rate of sedimentation remains at the same level throughout the 24 hours, or at any rate the decrease does not amount to more than 20% of the initial value.

"Type II. The rate of sedimentation markedly increases above the initial value in the course of the estimations and does not decrease to below the initial value.

"Type III. After having risen to above the initial value the rate of sedimentation again decreases gradually, often to far below the initial value."

Koster reported that the blood of 95% of 112 patients with cancer and of 100% of 14 cases of Hodgkin's disease conformed to one of these 3 types. He did not indicate the frequency with which each type occurred, nor did he report results on a definite number of patients with non-malignant conditions.

Feldman did not mention Koster's Type III in his report, and stressed maintenance of the initial sedimentation rate as an indication of cancer. Though he did not state a crucial decrease in the rate at which a line between positive and negative results could be drawn, he stated that maintenance of the initial rate occurred in 95% of 118 cases of malignant disease, and retardation occurred in 94% of 55 cases of non-malignant disease.

If the work of Koster and of Feldman could be confirmed, an important easily performed test for cancer would be available. We have therefore determined the sedimentation rates of a number of patients in whom the presence or absence of malignant disease had definitely been established, immediately after the withdrawal of blood and after it had been stored, and have attempted to evaluate the comparative sedimentation rates as a test for malignant disease.

Method. In the beginning of our work we attempted only to evaluate the comparison of initial rates with the rates after 24-hour storage as a test for cancer. Readings were made 1 hour after the tests were set up, and the sedimentation rate expressed in millimeters per hour. Sterile sodium citrate (3.8%), in the proportion of 1 cc. to 4 cc. of blood, was used as the anticoagulant; the samples were stored in sterile rubber capped vials at room temperature, which in our laboratory was approximately 31° C. The method duplicates the one used by Feldman, with the exception that serial determinations after storage at various periods of time were not made.

The test was recorded as positive if after 24-hour storage there was a decrease of less than 20% of the initial sedimentation rate, and negative if the decrease was more than 20%. For the results of the test scatter diagrams were constructed plotting the initial rate against the rate after storage, and statistical methods applied to the data if inspection of the diagrams suggested the indication for such treatment. It was realized that by the method used bloods displaying behavior of Koster's Type III would be missed.

The first few tests revealed marked discrepancy between our results and those previously reported. Since temperature seemed to be the only variable in the method which could account for the discrepancy, successive variations were made in the temperature of the blood during its storage and during the determination of the sedimentation rate. Altogether tests were run under the following sets of circumstances: (1) Rate determination and storage at room temperature, approximately 31° C. (2) Rate determination at room temperature, storage at 10° C. (3) Rate determination and storage at 20° C. This duplicates the room temperature at which Koster's tests were made. (4) Rate determination and storage at 10° C.

Later serial determinations of the sedimentation rate over a 24-hour period were made, so as to observe the incidence of Koster's Type III behavior. For these determinations the blood was stored and the rates determined at 10° C., since only at this temperature, as judged by the other tests, did there seem to be any difference between the behavior of bloods from patients with and without cancer. The serial rates were plotted as curves.

Results. 1. *Rate Determination and Storage at 31° C.* This method was used for 8 cases of cancer and 16 cases of non-malignant disease. The test was negative in all 24 cases (Fig. 1). A glance at Figure 1

reveals the fact that there was almost complete retardation of sedimentation in all cases.

2. *Rate Determination at Room Temperature, Storage at 10° C.* This method was used for 11 cases of cancer and 20 cases of non-malignant disease. The test was positive in 9 of the cancer cases, negative in 1, and equivocal in 1. It was positive in 16 cases of non-malignant disease, negative in 3, and equivocal in 1 (Fig. 2). A glance at Figure 2 shows that the initial rate tends to be maintained in all cases except those with very low initial rates, and that there is no difference between the behavior of blood from patients with malignant tumors and from patients with other diseases.

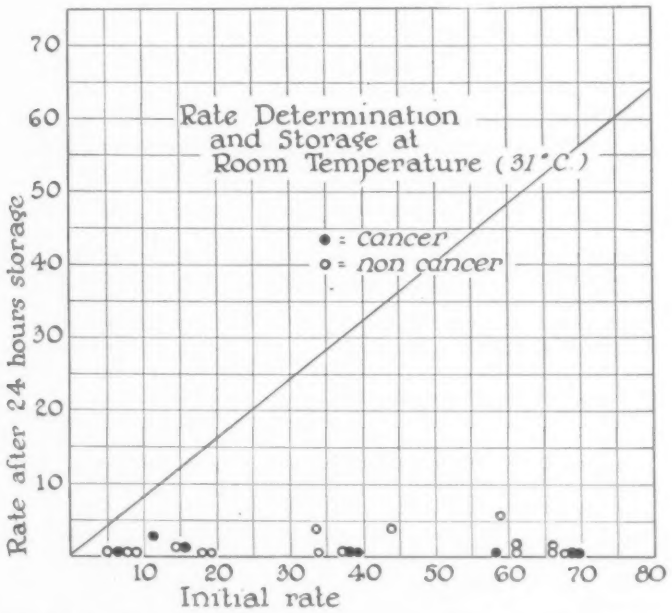

FIG. 1.—In this and in Figures 2, 3, and 4, "positive" tests lie above the diagonal line, and "negative" tests below it.

3. *Rate Determination and Storage at 20° C.* This method was used in 13 cases of cancer and 12 cases of non-malignant disease. The test was positive in 5 cases of cancer and negative in 8. It was positive in 3 cases of non-malignant disease, negative in 8, and equivocal in 1 (Fig. 3). In Figure 3 considerable scattering of the points is seen, indicating absence of the tendency for all tests to be negative, as in method 1, or positive, as in method 2, but there is no indication of difference in the behavior of bloods from patients with and without cancer.

4. *Rate Determination and Storage at 10° C.* This method was used for 31 cases of cancer and 39 cases of non-malignant disease. It was positive in 26 cases of cancer, negative in 3, and equivocal in 2. It

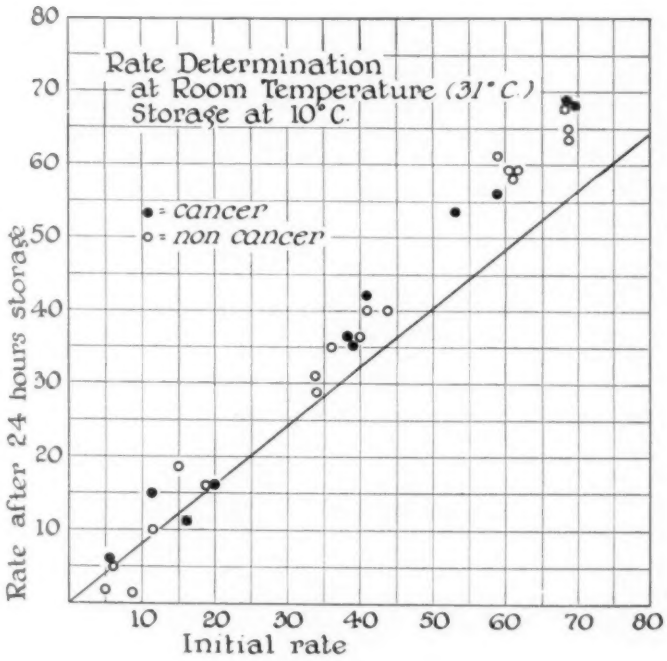
Rate Determination
at Room Temperature (31° C.)
Storage at 10° C.
● = cancer
○ = non cancer
Rate after 24 hours storage
Initial rate
10
20
30
40
50
60
70
80

FIG. 2

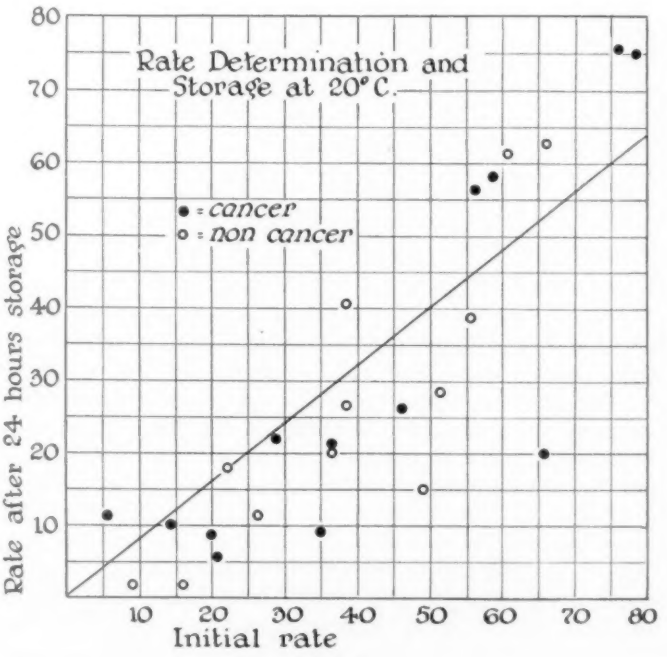
Rate Determination and
Storage at 20° C.
● = cancer
○ = non cancer
Rate after 24 hours storage
Initial rate
10
20
30
40
50
60
70
80

FIG. 3

was positive in 18 cases of non-malignant disease, negative in 17, and equivocal in 4 (Fig. 4). Statistical treatment of the data indicates that there is probably a significant difference between the mean percentile decrease in the sedimentation rates of the patients with and without cancer. As a test for cancer, the method gave approximately 85% correct, 10% false, and 5% doubtful results in the cancer cases; and 43% correct, 46% false, and 10% doubtful results in the non-cancer cases.

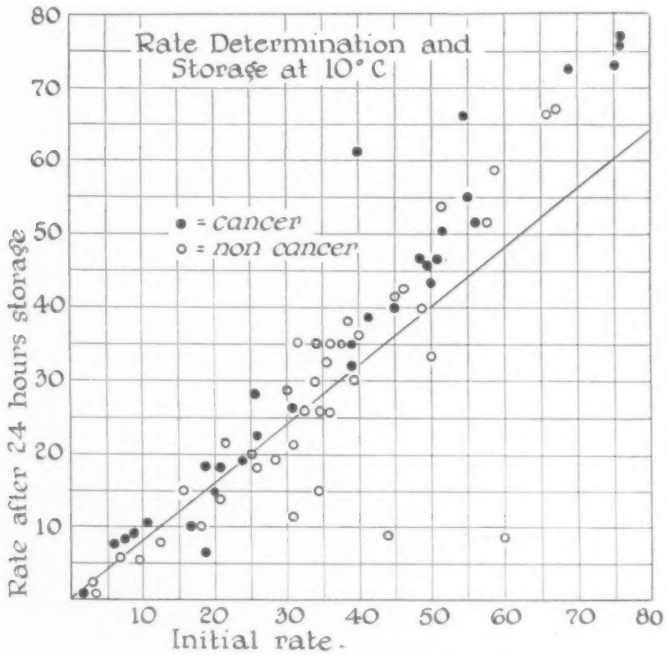

FIG. 4

5. *Serial Determinations, After 1, 2, 3, 4, 5, 6, and 24 Hours. Rate Determination and Storage at 10° C.* This method was used in 33 cases, 17 of cancer and 16 of non-malignant disease. In 5 of the cancer cases there was a definite increase in the sedimentation rate, amounting to 3 mm. or more at one time or another, and in 8 others there was a slight increase, amounting to less than 3 mm. (Fig. 5). However, in all of these 13 cases, the decrease in the rate after 24-hour storage was less than 20%, so that none fell into Koster's Type III. In 1 non-cancer case there was a definite increase, and in 8 a slight increase (Fig. 6). In all of these cases the decrease after 24-hour storage was less than 20%; they all therefore gave false positive reactions.

Discussion and Summary. Our results demonstrate that retardation or maintenance of the initial sedimentation rate in stored blood is capricious, and depends in large part upon the temperature of the

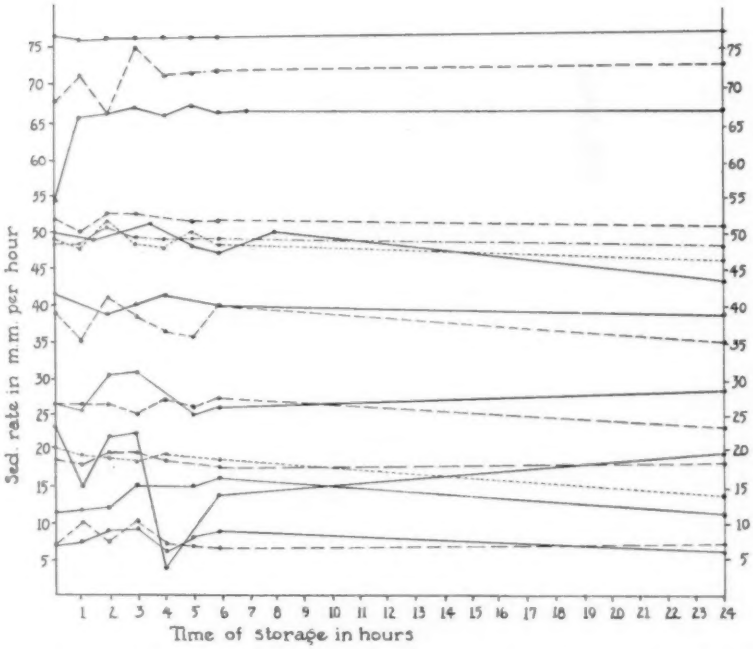

FIG. 5.—Serial sedimentation rates—cancer cases. In this and in Figure 6, solid and broken lines are used so that individual curves may be followed easily.

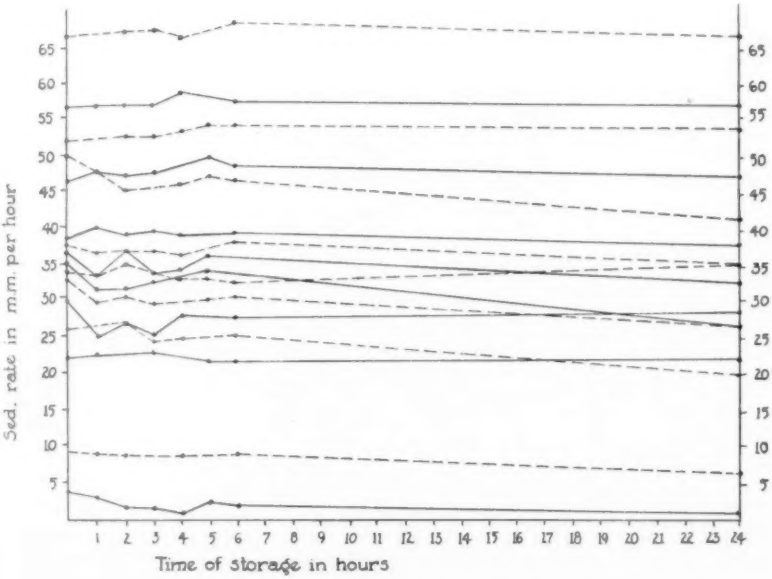

FIG. 6.—Serial sedimentation rates—non-cancer cases.

blood when the rates are determined and at which it is stored. We were able to find only one set of temperature circumstances at which there was significantly less retardation of the mean sedimentation rate in cases of cancer than in non-malignant diseases. When blood was stored 24 hours at 10° C. and the rates determined with the blood at the same temperature, the rate remained within 20% of the initial rate in about 4 cancer cases out of 5, and decreased more than 20% in a little less than half of the cases of non-malignant disease. One would therefore expect that in an unknown case a positive test would indicate that the chances of cancer being present are about 2 out of 3, while a negative test would indicate that there is only 1 chance in 5 that cancer is present. It is obvious that a negative test would give more information than a positive one, but that in either instance the test would be far from reliable. There is a strong probability, suggested principally by the high incidence of positive tests in persons without cancer, that maintenance of the initial rate is not related at all to the presence of cancer. The capriciousness of the test also suggests the possibility that the seemingly significant differences were due entirely to chance. This possibility is strengthened by the fact that the test is empirical, and cannot be correlated with any known differences between the blood cells or plasma of persons with and without cancer.

Conclusions. By the methods which we used, maintenance of the initial sedimentation rate in blood stored for 24 hours was not a reliable criterion for the presence of malignant disease.

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THE PROGNOSIS OF UNTREATED PATENT DUCTUS ARTERIOSUS AND THE RESULTS OF SURGICAL INTERVENTION

A Clinical Series of 50 Cases and an Analysis of 139 Operations

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Since the first successful ligation of a patent ductus arteriosus by Gross and Hubbard in 1938,[4] we have been concerned with the problem of recommending surgery for such patients. During years of observation of a group of patients with patent ductus arteriosus, we were impressed with the apparent innocuousness of this lesion. Children observed through their entire school careers were able to carry on regular school activities with no restrictions and showed no evidence of cardiac disability at any time. Young men with this lesion have

worked at hard labor with no symptoms of cardiac strain. Women have borne children with no signs of heart failure. Such observations do not arouse enthusiasm for surgical intervention and we felt it necessary to carry on further studies before recommending such radical treatment. It seemed desirable to learn what could be expected in regard to longevity and cause of death in untreated cases of patent ductus arteriosus and to examine the record of surgery to date.

For these purposes three sets of data were assembled. Autopsy records on adults with patency of the ductus arteriosus were collected from the world literature and our own files; the analysis of these cases has been published elsewhere.[12] The clinical histories of our own patients, many of whom were observed for years, provide very useful data. Finally, the generous aid of surgeons and internists throughout the United States and Canada has provided us with clinical and surgical records on the majority of all the patients who have so far been operated upon to ligate the open ductus. The present paper is a report on our own experiences and on the surgical results to date.

Personal Observations. Recently we reported[13,15] the clinical findings on 23 patients with patent ductus arteriosus. Most of them had been observed over a period of years. At that time none of these patients had died and none had developed subacute bacterial endarteritis or serious cardiac failure. This was a young group, the average age being 20.3 years. With one exception they were well developed physically but most of them showed enlargement of the pulmonary artery and the hilar vessels. In only 4 instances was there evidence of progressive cardiac enlargement. Three of the latter patients were operated upon.

The first ligation in our series was attempted in a boy of 9 but the vessel ligated was probably not the ductus arteriosus. The physical signs were unchanged as a result of surgery and the boy's status was neither improved nor impaired. The second patient, a young man of 19, died as a result of rupture of the ductus at operation. The third operation, on a 10 year old girl, was a complete and impressive success.

Since the last report our series of patients has increased to 51, partly by new patients, partly by the follow-up of patients who had not been seen for some years. Thirty-seven of these patients have been thoroughly studied by physiologic methods devised in our laboratories.[8–11] Using criteria established elsewhere,[6,7] at least 20 of these patients might have been subjected to surgery. We chose, however, to delay surgical intervention until we could complete our studies and have only sent 3 additional cases to surgery. Three of these operations were apparently entirely successful; the fourth was Case 4 cited below.

The observations and experiences with these patients emphasize two features which point the opposing arguments about surgery. We shall illustrate by a few brief case notes:

Case Studies. CASE 1. A 58 year old male was admitted to the surgical service of Dr. O. H. Wangensteen with a diagnosis of carcinoma of the stomach. On physical examination evidence of patent ductus arteriosus was found and this was confirmed by careful physiologic studies. However, there was no

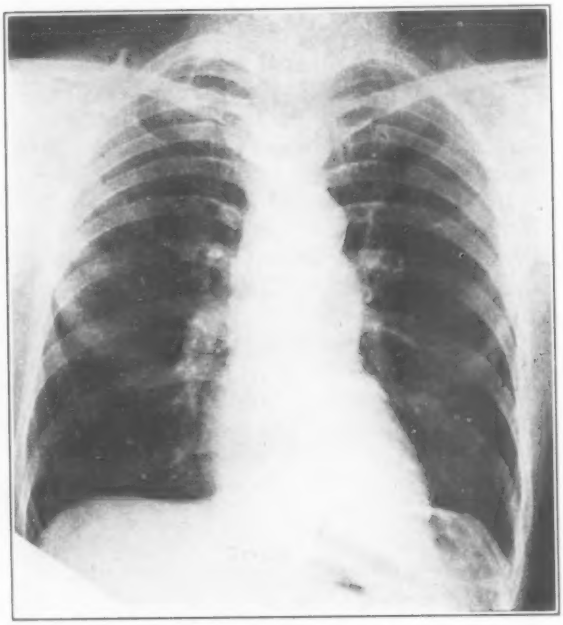

FIG. 1.—Teleroentgenogram of heart.

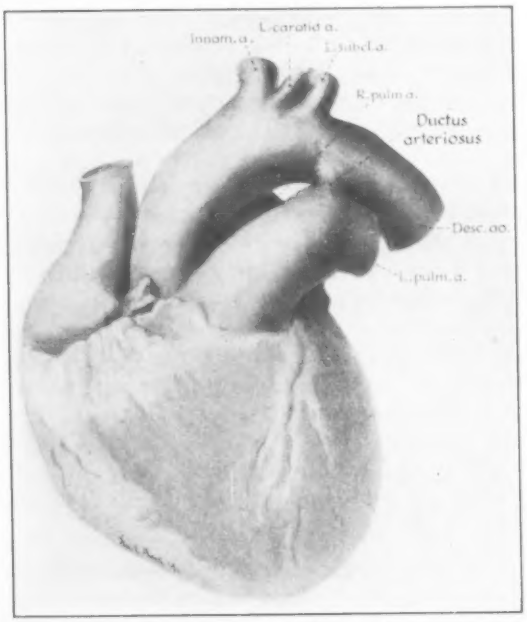

FIG. 2.—Drawing of the specimen.

indication of cardiac disability in spite of the fact that the patient had done hard manual labor all his life. He was subjected to total gastrectomy and withstood this severe surgical procedure without signs of a subnormal cardiac reserve. He eventually died of non-cardiac complications and postmortem revealed an enlarged heart and an open ductus arteriosus. Figure 1 shows teleroentgenogram of the heart and Figure 2 is a drawing of the specimen.

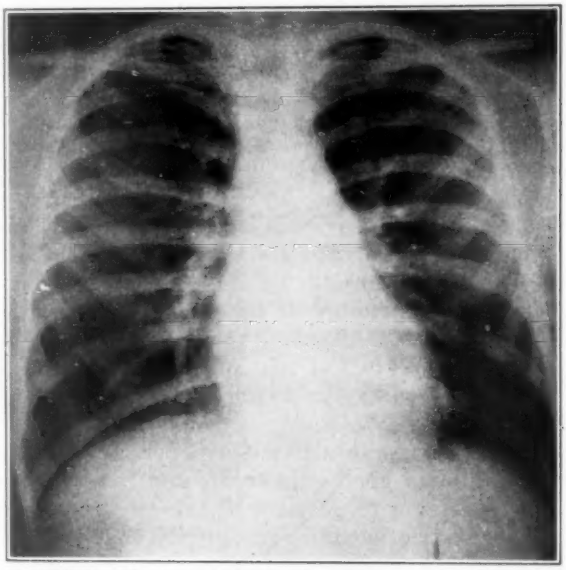

FIG. 3.—Case 2. Patent ductus arteriosus, with enlargement of the pulmonary artery age 10.

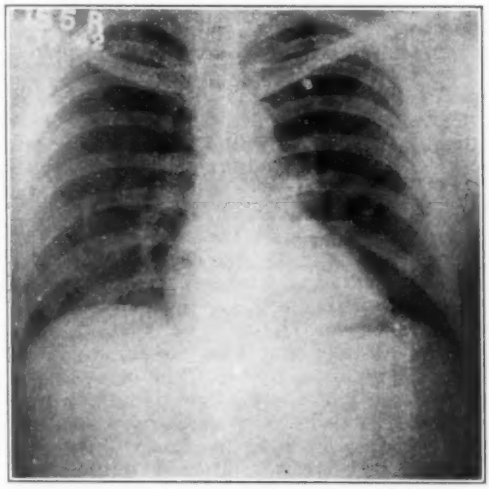

FIG. 4.—Same patient at age of 28. Still signs of enlargement.

CASE 2. A 28 year old male was examined after he had been rejected for military service because of "heart disease." We had examined him at the

age of 10 and had recorded then a diagnosis of patent ductus arteriosus with considerable enlargement of the pulmonary artery (Fig. 3). In the intervening 18 years he had felt himself to be perfectly well and was able to carry on very heavy manual work, including service in two C. C. C. camps. He had even passed an examination for life insurance. On reëxamination the evidence for patency of the ductus was undeniable. His heart was slightly enlarged and there were still signs of enlargement of the pulmonary artery (Fig. 4). He was free from symptoms and complaints and was unusually well developed and muscularly powerful.

CASE 3. A woman of 30 was first informed that her heart was not normal when she was examined incidentally to pregnancy. She exhibits all signs of typical patency of the ductus arteriosus. She has never had any symptoms or cardiac complaints. She went through labor without difficulty and in the 2 years since her baby was born she has done hard work with no cardiac embarrassment.

Obviously such observations do not indicate the necessity for surgery. Further argument for conservatism is suggested by the fact that 2 of our patients who have been followed for years have recently shown evidence of spontaneous closure of the ductus and disappearance of all signs of any lesion. The details of these 2 remarkable cases will be presented elsewhere. We believe that spontaneous late closure is very rare but it cannot be excluded as a remote possibility in the prognosis of any individual case.

On the other hand, our own recent experiences confirm the belief that a present status of good adjustment and freedom from cardiac disability in a patient with patency of the ductus arteriosus is no guarantee that subacute bacterial endarteritis may not intervene at any time or that cardiac failure may not eventually appear. Histories on 3 patients may be cited.

CASE 4. This 53 year old woman had always known that her heart was abnormal but had led a reasonably normal life without real cardiac disability until 6 months before death. Slowly progressive fever and weakness continued after a "cold" for several months before cardiologic consultation resulted in the diagnosis of patent ductus arteriosus and subacute bacterial endarteritis. By the time consent for surgery was obtained the woman was practically moribund and she died an anesthetic death on the operating table before surgery was started. Postmortem examination fully confirmed the diagnosis and revealed a patent ductus that would have provided a minimum of operative difficulty for ligation had this been attempted before her final illness.

CASE 5. The diagnosis of patent ductus arteriosus was made in this 16 year old girl when she was 7 years of age. She was subsequently carefully studied and was regarded as a proper candidate for ligation but her general freedom from cardiac embarrassment made it appear that there was no immediate necessity to insist on surgery in the face of parental unwillingness. A month before death she had apparently been in her normal health when she suddenly complained of dizziness, chills, and fever. She died in 4 weeks of subacute bacterial endarteritis with pulmonary infarcts, vegetations in the pulmonary artery and valve and a heavy infection of *Streptococcus viridans*. We were only apprised of these developments by the family physician after the patient expired. Our original diagnosis was confirmed at autopsy.

CASE 6. This 46 year old woman came in for consultation from her office work with complaints of recently developed dyspnea and palpitation. She had been told early in life that she had a cardiac disorder but this had never seriously inconvenienced her before. At examination she was in well-marked

failure but no murmurs could be heard. She died in failure within 2 weeks. At autopsy the heart was found to be enlarged and there was a large patent ductus arteriosus with no other defects.

Reports in the Literature. A study of the recent literature failed to give us data which could be used to predict what could be expected in patients with patent ductus arteriosus who receive no treatment. We found it necessary, therefore, to review the entire medical literature and we have analyzed all the cases of patent ductus arteriosus in adults from which postmortem examinations have been reported. The details of this study are given elsewhere.

Briefly, it may be stated that 80% of such patients eventually succumb to their cardiac lesion. These patients who were alive at 17 years of age averaged 35 years of age at death. At least 40% of these patients died of subacute bacterial endarteritis and most of the remainder died of congestive cardiac failure. Spontaneous rupture of the pulmonary artery or the ductus occurred in a few cases.

The age distribution of our own patients likewise indicates that the life expectancy of persons with untreated patency of the ductus arteriosus is considerably shortened. It is true that the majority of our patients were found in a children's clinic but we have made considerable efforts to find adult cases with no great success. The average age of the entire series of 51 patients is less than 25 years and the oldest patient in the series died at 58. These data from the literature and our own series force a reconsideration of a conservative attitude toward what might seem radical surgery.

The Surgical Record. From the current literature, but mostly through personal correspondence with individual surgeons and internists, we have obtained and analyzed the data on nearly all patients on whom ligation of the ductus has been attempted. The record is surely not complete but we are certain that it contains the great majority of operations done at the time of writing.

One hundred and forty patients have been operated upon by a total of 25 surgical teams. Of these cases, 107 were uncomplicated, while operation was performed on 33 patients in the presence of subacute bacterial endarteritis. Seven of the patients were in our own series.

In the 107 uninfected cases, 81 operations were completely successful, that is, the murmur and thrill disappeared and the pulse pressure decreased. In a number of these successful cases, a distant short systolic murmur remained over the pulmonic area. It is our impression that this murmur results from the persistent dilatation of the pulmonary artery and not from persistent patency of the duct. In 14 instances, however, the continuous machinery murmur was not permanently obliterated after surgery. The murmur returned in from a few weeks to several months after operation. It is quite probable that in these 14 instances, there was a recanalization of the duct due to failure of the ligature material to hold. It is obvious that further research must be carried on in order to find the perfect ligature material and method of ligation.

In 6 cases, death resulted from rupture of the duct at operation.

From recent studies, it is quite certain that this accident will not occur as frequently in the future. Surgeons have already perfected their methods to avoid this complication. In 2 instances, subacute bacterial endarteritis developed after operation. This is rather disturbing but it is reasonable to assume that the rest of the patients who have been operated upon and who have been followed over a period of months or years will probably not develop infection as result of the surgery. In 1 case, death resulted from infection of the surgical wound followed by mediastinitis. In another case, that of a physician 39 years of age, a direct arteriovenous communication was found between the pulmonary artery and the aorta. Fortunately, the surgeon had the good judgment to terminate the operation without attempting ligation. The patient is none the worse for his experience. A study of patent ductus arteriosus in the adult reveals reports of a number of instances of arteriovenous fistulæ too short to ligate. At present it is impossible to diagnose such a direct communication and there will be other instances when surgeons will meet with this inoperable situation.

A vessel other than the ductus was ligated in 3 cases. In 1 instance the aorta was mistaken for the duct and, of course, ligation resulted in death. In another instance, a strand of fibrous tissue was mistaken for the duct and this patient also died as the result of the operation. The third case resulted in no harm to the patient.

It is a remarkable fact that errors in diagnosis were made in only 2 instances in the entire group of 140 patients. It is, therefore, apparent that the diagnosis of patent ductus arteriosus can be made with security. The old idea that it was hazardous to tie off the duct because the patent ductus might be acting as a safety shunt for other congenital cardiac lesions need no longer be seriously considered. In those instances where the ductus does act as a safety shunt there are always complicating congenital developmental defects which should not be difficult to differentiate from the simple uncomplicated patent ductus arteriosus. Nine deaths have resulted from these 107 operative cases; a mortality rate of 8.5%.

Of the 33 patients who were operated upon in the presence of subacute bacterial endarteritis, 20 resulted in apparent complete success; that is, the blood cultures became sterile and the patient's general condition improved markedly. Some of these patients have been followed only a period of months, not long enough to draw definite conclusions, but some have certainly been restored to completely normal health. Five patients died on the operating table as the result of hemorrhage. In 8 instances, the fever persisted in spite of the ligation of the duct. Somewhat more than 50% apparent success in a group of cases in which previously 100% mortality was to be expected can be considered an excellent result.

Discussion. The data in this paper were assembled in the hope that practical conclusions would emerge from their analysis. We believe that certain conclusions are, in fact, clearly indicated and that some recommendations can now be made with confidence. Surgery is advisable in the great majority of patients with patent ductus arteriosus with or without subacute bacterial endarteritis. In the latter case

surgery affords more than a forlorn hope. The uninfected patient cannot expect a normal life span without surgery and is liable to develop subacute bacterial endarteritis at any time in spite of present apparent well-being.

The experience of Touroff and other surgeons operating in the presence of subacute infection shows that ligation should be attempted immediately when the diagnosis is made. The continued presence of the infection renders the operation much more hazardous because of increasing edema and friability of the ductus and adjacent tissues. Moreover establishment of vegetations on the heart valves creates pockets of infection which may persist after the abnormal shunt of blood is stopped. In a number of cases ligation in these patients brought about temporary amelioration but persistent bacterial endocarditis had already been established and the patients eventually succumbed. It seems certain that sulfa drugs, fever therapy and transfusions have only a delaying action at best. Trial of a course of such treatment before surgery is only to increase the risk of delay.

Successful ligation of the uninfected duct will prevent congestive heart failure. However, it cannot be stated with certainty that the danger of subacute bacterial endarteritis is entirely removed by ligation. It is reasonable to hope at least that the liability to infection will not be increased by surgery. Definite conclusions must wait until these operated patients have been observed for years. In 2 cases subacute bacterial endarteritis developed after surgery. In 1 of these the duct had recanalized. Even without recanalization it must be admitted that the blind pouches in the aorta and pulmonary artery represent favorable sites for the development of infection in the same way that any cul-de-sac in an area of exceptionally turbulent blood flow is a potential spot for infection. However, we can believe that the remaining pouches are less dangerous than the open ductus in this regard. For one thing, the persistently open ductus almost always leads to the development of atheromata and roughening of the wall of the pulmonary artery and thus creates a specially vulnerable region. Finally, if turbulence *per se* is favorable to infection then the ligated ductus is much safer; the extent of turbulence can be gauged roughly by the intensity of the murmur.

The incidence of subacute bacterial endarteritis is small until the patients are in their teens and the highest incidence is in the third decade of life. It might be suggested therefore that there is slight danger in delaying surgery in very young children. This is not entirely true. The incidence of infection in young children is not entirely negligible and the difficulty of the surgery itself is increased with increasing age. Progressive enlargement of the pulmonary artery and possible relative shortening of the ductus tend to obscure and to reduce the safe field of operation. The rapidity of recovery of young children from the operation is astonishing.

The diagnosis of patent ductus arteriosus can be made with great assurance. We have noted that in the entire series of 140 operations, only 2 diagnostic errors were made. In both of these the data collected by the internists were incompatible with the diagnosis.

Very rarely there may be a direct open communication between the ascending aorta and the pulmonary artery. Fraentzel,[2] Oberwinter,[14] and Girard[3] have reported such cases. These would be indistinguishable from a true patent ductus but would, of course, be inoperable. More frequently, but still only rarely, the ductus may be so short as to be inoperable; it may even be reduced to a hole between aorta and pulmonary artery at the normal ductus site. We must depend on the ability of the surgeon to recognize such a condition in the surgery and to retreat without pursuing a dangerous dissection.

TABLE 1.—SUMMARY OF THE RESULTS OF SURGICAL INTERVENTION IN PATIENTS WITH PATENT DUCTUS ARTERIOSUS

Total number surgeons	25
Total number cases operated	140
Number uninfected cases	107
1. Completely successful	81
2. Continuous murmur persists	14
3. Rupture of duct at operation	6
4. Operation followed by subacute bacterial endarteritis	2
5. Infection of wound and mediastinitis	1
6. Found inoperable	1
7. Vessel ligated not ductus arteriosus	3
8. Error in diagnosis	2
9. Total number dead after operation	9
Cases operated upon with subacute bacterial endarteritis	33
1. Completely successful	20
2. Died at operation	5
3. Fever persists after successful ligation	8

The low mortality rate indicated in Table 1 is gratifying and surprising in view of the serious nature of the operation. It is a tribute both to the skill of the individual surgeons and to the development of thoracic surgery in recent years. It is necessary to emphasize, however, that both skill and extensive experience in thoracic surgery are required to undertake the operation. This is exemplified by the fact that two surgeons, Dr. Robert Gross,[5] of Boston, and Dr. John C. Jones,[7] of Los Angeles, have done more than half of all the ligations to date with mortality rates less than half that for the other surgeons who have devoted less attention to this particular operation. In general, we can expect that accurate diagnosis and skilled surgeons can guarantee us a mortality of about 5% in uninfected cases.

Summary and Conclusions. 1. Diagnosis of patent ductus arteriosus can be made with great certainty, and complications making surgical ligation inadvisable are readily recognized.

2. The great majority of patients with this defect suffer no serious disability or restriction of activity during most of their lives, but their life expectation is greatly shortened by the continued presence of the defect.

3. Experience to date shows that ligation of the uninfected ductus can be made with a mortality of less than 10%.

4. Ligation of the ductus in the presence of subacute bacterial endarteritis offers an even chance of survival in the face of practically certain death without ligation.

5. The danger of development of subacute bacterial endarteritis after successful ligation cannot properly be estimated as yet.

6. Six case histories are cited which illustrate opposing arguments for and against ligation.

7. An analysis is presented of the results of 140 operations to ligate the ductus.

8. It is concluded that the majority of patients with patency of the ductus arteriosus should be sent to surgery for ligation after careful clinical studies have been made on them.

9. Ligation should be attempted immediately if subacute bacterial endarteritis develops.

10. Ten patients with uninfected patent ductus arteriosus have been operated upon by Dr. O. N. Wangensteen at the University of Minnesota Hospital, the last 8 cases with complete success. As far as is known, in none of them has there been a recurrence of signs indicating recanalization.

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CLINICAL SIGNIFICANCE OF HYPERVENTILATION

THE RÔLE OF HYPERVENTILATION IN THE PRODUCTION, DIAGNOSIS AND TREATMENT OF CERTAIN ANXIETY SYMPTOMS

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Patients with anxiety frequently come to the physician complaining of dyspnea, giddiness, fainting and coldness of the extremities. The physician usually recognizes that no organic disease is present, but he is unable to explain to the patient the manner in which anxiety has

produced the symptoms. In recent years the physiologists have called attention to various mechanisms by which afferent stimuli arising from the emotional content of thought or from various sensory nerves can produce changes in function. The clinician has gradually realized that reflex disturbance in function is the physiologic basis of many of the symptoms of which his psychoneurotic patients complain. It is well known that sensory stimuli can cause abdominal pain by reflex contraction of the stomach or colon. Studies on the carotid sinus reflex have demonstrated that sensory stimuli can cause symptoms by cardiac standstill or marked lowering of the arterial pressure. The purpose of this paper is to point out how afferent stimuli arising from the emotional content of thought or from sensory nerves may affect the respiration and thereby produce clinical symptoms.

Normal respiration is controlled by both nervous and chemical factors. Although in the past many textbooks have stressed the importance of the chemical factors, it is now recognized that many of the variations in breathing occurring in everyday life are due to reflex stimuli.[2] The afferent impulses arise from moving muscles, various viscera, eyes and ears, any sensory nerve ending, and particularly from the emotional content of thought. The lay public has long realized that emotions influence the respiration. The breath is held during a moment of suspense, and the respiratory rate is increased with sexual excitement. Finesinger[4] has studied the effects of pleasant and unpleasant thoughts on the respiration and has demonstrated this type of respiratory control in the laboratory.

When an individual's respiration is increased by sensory or emotional stimuli, he may complain either of dyspnea or of certain other symptoms, such as giddiness, light-headedness or fainting, which are secondary to the disturbance in cerebral metabolism brought on by the overbreathing. When the respiration is increased by a reflex mechanism without a corresponding rise in cerebral metabolism, the carbon dioxide content of the alveolar air is diminished and, because of the rapid diffusibility of that gas, a rapid loss of carbon dioxide from the body occurs. This results in alkalosis, and associated with this there is an alteration in cerebral metabolism caused either by the increased alkalinity of the arterial blood or indirectly by decreased cerebral blood flow. The disturbance in cerebral metabolism is shown by the development of cerebral symptoms and by the appearance of bizarre waves in the electroencephalogram.[3] The rise in pulmonary ventilation secondary to exercise does not produce alkalosis because the increased loss of carbon dioxide through the lungs is balanced by the increased production of carbon dioxide and organic acids as a result of the increased metabolism.

The ease with which cerebral symptoms are precipitated by voluntary hyperventilation and the order in which they occur are subject to considerable variation.[2,5,6,9,11,13] They have been described as a feeling of light-headedness, unsteadiness, faintness, or a far-away feeling. This sensation is followed by numbness and tingling of the tongue, mouth and extremities. The hands become cold and pale

and a tremor may appear. If the subject is standing, syncope may occur. Tetany occurs as a late symptom with tightness of the muscles, increased reflex irritability, and finally carpopedal spasm. The Chvostek sign may become positive. The mental reactions of the subjects vary tremendously; some have almost no change, others may become excited, weep and complain bitterly of the symptoms produced, and may have difficulty in stopping the procedure. On rare occasions rebreathing into a paper bag may be necessary to stop the hyperventilation.

When a normal subject hyperventilates voluntarily, he recognizes the cause of his symptoms and is not alarmed; but the patient who hyperventilates unknowingly develops the symptoms without apparent cause and he, as well as his physician, may fear that they indicate the presence of serious organic disease. The symptoms of which the patient complains may be attributed to overbreathing if they are produced by forced respiration and if they disappear on holding the breath. The initial symptoms appear with equal ease in the normal subject and in the patient. The significant fact is that the symptoms which bring the patient to the physician can be faithfully reproduced in the patient by the forced breathing.

In the last 3 years it has been our custom to determine the effects of hyperventilation in patients who (1) complain of light-headedness, giddiness, far-away feeling, feeling of unreality, numbness of face, mouth and hands, carpopedal spasm, and fainting; (2) complain of dyspnea in the absence of demonstrable signs of congestive failure. The patients studied complained of the same symptoms which occurred in normal control subjects during forced breathing.

To test the response to hyperventilation the patient is instructed to breathe deeply and rapidly. Frequently it is advisable for the physician to demonstrate what is desired, and during the test the patient must be encouraged repeatedly to continue breathing vigorously and not relax his efforts. If the problem is one of fainting, the test should be carried out in the upright position, for it is only rarely that loss of consciousness develops in the recumbent position. In the majority of instances a 2 or 3 minute period of vigorous hyperventilation is sufficient. The physician observes the objective effects while the patient is breathing deeply, and when he feels that there has been adequate hyperventilation he questions the patient about his symptoms.

Cerebral Effects of Hyperventilation. The history of patients who faint from hyperventilation is fairly characteristic. They frequently feel light-headed and giddy when lying down and notice numbness of the face and extremities. They think they are going to lose consciousness but rarely do so. The occurrence of symptoms when the patient is in the recumbent position rules out postural hypotension as the cause of the fainting. When sitting and standing they have many of these abortive attacks which, from time to time, are followed by the loss of consciousness. Syncope results from the combination of the decrease in cerebral blood flow produced by pooling of blood in the lower extremi-

ties and the disturbance in cerebral metabolism caused by the alkalosis. In the last 3 years 20 patients with this syndrome have been observed by us. The following case history is illustrative of this group.

Case Studies. CASE 1. An 18-year old student nurse was admitted to Grady Hospital because of repeated attacks of syncope. In the past she had fainted occasionally, but never often enough to cause her any concern. Recently, however, syncope had occurred so frequently that she was afraid of being dismissed from training school. The loss of consciousness occurred only when she was sitting or standing, never when she was lying down. Before losing consciousness the patient felt light-headed and, as she described it, had a far-away feeling. Her hands and feet were cold, and there was a tingling sensation about her mouth, down the arms, and down the inner surfaces of her thighs. Frequently the prodromal symptoms were not followed by syncope. They occurred regardless of whether the patient was standing or lying down, although syncope did not occur when the patient was recumbent. Consciousness returned quickly after the patient fell to the floor. Recovery was complete within a few minutes, and the patient would return to work immediately thereafter. There was no history of biting the tongue or of incontinence of urine or feces. The patient had always been healthy. She performed her work with ease and participated in sports without difficulty. The routine physical examination and laboratory findings were normal.

Pressure on either carotid sinus did not cause faintness or marked change in pulse rate, indicating that carotid sinus irritability was not responsible for her fainting. On standing motionless for several minutes the pulse rate increased normally and there was only a slight narrowing of the pulse pressure, ruling out the possibility of postural hypotension. The patient was then instructed to sit upright in a chair and breathe as deeply as possible. After a few breaths she complained of giddiness, blurred vision, numbness and tingling of the arms and legs, and then became unconscious. The heart rate was rapid but the pulse pressure remained unchanged. The hands and feet were cold. The patient was placed in the horizontal position and after a few seconds of apnea, consciousness was recovered. The patient stated that this fainting attack was exactly like the ones which she had suffered previously. Hyperventilation was then carried out in the recumbent position. Giddiness, feeling of unreality, numbness and tingling of the face, arms and legs and coldness of the hands and feet resulted, but the patient did not lose consciousness.

Case Comment. This patient was an anxious young woman who responded to a variety of stimuli with a reflex increase in respiratory rate. The overbreathing caused alkalosis and produced a giddy, uncertain feeling. This sensation frightened her and caused further increase in pulmonary ventilation with the accentuation of the cerebral symptoms. Numbness and tingling developed around the face and in the extremities, causing still greater anxiety. If the patient was recumbent, the disturbance in cerebral metabolism caused by the alkalosis did not become severe enough to cause loss of consciousness. The fainting episodes embarrassed the patient. Every time that she felt slightly giddy, she immediately became afraid that she would faint. This caused more overbreathing and at times syncope.

The mechanism of the attacks of syncope was explained to the patient. She was told that because of her own biologic makeup and personality structure a variety of stimuli would continue to cause a reflex increase in pulmonary ventilation and that when this occurred she would experience the sensations of giddiness and light-headedness. She was told that these sensations were a normal response to over-

breathing, were physiologic rather than pathologic, and would disappear if she held her breath. The patient has had no further difficulty. She interprets correctly the cerebral symptoms of overbreathing so that they no longer cause anxiety and the vicious cycle has been broken.

Though the emotional control of thought is the most common stimulus for overbreathing, sensory impulses from any part of the body may be responsible for the hyperventilation. In the following case history afferent stimuli from the bladder produced the overbreathing.

CASE 2. An elderly colored man with a moderate degree of hypertension, but no signs of cardiac decompensation, was admitted to the Peter Bent Brigham Hospital because of a chronic urethral stricture and an irritable, infected bladder. Bladder drainage was accomplished by an inlying urethral catheter. Later in the day the patient began to breathe deeply, and shortly afterward became unconscious. At first it was thought that he had developed cardiac asthma, but examination failed to reveal evidence of cardiac decompensation. Then it was noted that the tube leading from the catheter was twisted, causing the flow of urine to become obstructed. As soon as the bladder was emptied, the respiration became much quieter and the patient regained consciousness. Similar episodes occurred several times during his hospitalization.

Case Comment. These 2 patients are similar in that their symptoms were the result of hyperventilation. They demonstrate that the increased respiratory rate may be due to a variety of causes, and the presenting symptoms may be any of the symptoms of overbreathing.

Hyperventilation With Dyspnea. Certain patients with an adequate circulation come to the physician complaining primarily of dyspnea. This may be present at rest, on slight exertion or on awakening at night. A carefully taken history frequently reveals that the dyspnea is accompanied by other manifestations of hyperventilation such as dizziness, giddiness, light-headedness, numbness of hands and feet, coldness of the extremities and drawing of the hands. During the last 3 years we have observed 15 patients in whom the dyspnea of anxiety was mistaken for the dyspnea of congestive failure. These patients had no evidence of circulatory insufficiency and their symptoms could be reproduced by a short period of hyperventilation. Some of these patients had no signs of any organic disease; others had hypertension or rheumatic heart disease with no evidence of cardiac decompensation. The following case history is typical of this group.

CASE 3.—A 44-year old housewife complained of dyspnea on exertion and of paroxysmal nocturnal dyspnea of 4 years duration. She had been told that she had an elevated blood pressure and albuminuria. On many occasions the attacks of nocturnal dyspnea had been severe enough to require the administration of morphine. The patient had a difficult home situation and had been to a number of doctors in the last few years because of headache and neurodermatitis. The arterial pressure was 160/110 mm. Hg. Otherwise the physical examination was negative. The urine contained 2+ albumin. The phenolsulfonphthalein excretion was within normal limits. Roentgenographic examination showed that the heart was at the upper limits of normal in size with a prominent left ventricle. The lungs were clear, and there was no edema. The vital capacity was normal.

The patient was given digitalis and told to return in 1 week. She knew that digitalis was prescribed for heart disease and that she had convinced her doctor

that she had heart disease. During the next day the patient became progressively more dyspneic. Late that afternoon she was admitted to the Peter Bent Brigham Hospital. The patient was breathing deeply and rapidly, the hands were cold and there was bilateral carpopedal spasm. She complained of numbness of the face and extremities. After rebreathing into a paper bag for a few minutes, the patient became quiet and the dyspnea disappeared. Further questioning revealed that the patient had had repeated attacks of dizziness and light-headedness during the preceding 4 years. The attacks of nocturnal dyspnea were all accompanied by cold hands and feet, light-headedness and numbness of the face and extremities. It now seemed clear that she had never had any symptoms of heart failure, but that all her symptoms could be attributed to reflex hyperventilation.

The mechanism by which anxiety caused an increase in respiratory rate was explained to the patient. She accepted the fact that she did not have heart disease, but later her anxiety expressed itself as globus hystericus and pain in the abdomen.

Comment. These experiences stimulated our interest in patients with apparently compensated heart disease who complained of dyspnea. Patients with mitral stenosis without any objective sign of congestive failure were examined. These subjects had normal vital capacities, but had complained of dyspnea for years. In some of these subjects voluntary hyperventilation produced a typical anxiety panic. They interpreted the feeling of light-headedness and numbness in the face and extremities as symptoms of heart disease. Questioning revealed that the dyspnea of which they had complained was accompanied by cold hands and feet, light-headedness, and numbness and tingling. Exercise tests showed that these patients were no more dyspneic on exertion than normal subjects.

It gradually became clear that the diagnosis of cardiac disease evoked some anxiety in every patient. If this anxiety caused overbreathing, the patient invariably interpreted the resulting symptoms as an indication of cardiac failure. It is helpful if the patient realizes that many things other than heart failure or cerebral disease can cause the sensations of unreality, giddiness, and dyspnea. This is accomplished by having every patient with compensated heart disease overbreathe during the course of the physical examination. The patient is then questioned about his symptoms and his interpretation of them. The underlying physiologic mechanism producing the symptoms is then explained.

At least two different mechanisms appear to be operative in the dyspnea of anxiety. In the first type described here there is a marked increase in pulmonary ventilation. This increase in respiratory rate may result solely from emotional stimuli, or it may be an exaggerated response to some somatic stimulus, such as walking slowly, which would normally cause only a slight increase in pulmonary ventilation. In the second type, not discussed in this paper, the patient is conscious of the effort in breathing at rest. He may complain of breathlessness even when there is little actual change in pulmonary ventilation.

Hyperventilation is of importance in other conditions. In many patients with epilepsy, petit mal attacks can be precipitated by over-

breathing.[7] Grand mal attacks are not produced in this manner. Various authors have pointed out that many of the symptoms of neurocirculatory asthenia are caused by hyperventilation.[1,8,14] Probably the hyperventilation is a sign of the underlying psychoneurosis and therefore it is the result rather than the cause. Recent studies have suggested the importance of hyperventilation in aviation medicine.[10,12]

The average physician pays little attention to the physiologic mechanisms underlying the complaints of his psychoneurotic patients. The symptoms are real to the patient, but they frequently remain vague and indefinite to the doctor. Clinical observation and study of the physiologic changes underlying these symptoms will be of benefit to both patient and physician.

Summary and Conclusions. 1. Respiration is controlled by both, reflex and chemical mechanisms. The variations in respiration which occur during our daily routine of living are caused chiefly by reflex phenomena. Afferent stimuli from any organ in the body or from the emotional content of thought may cause the pulmonary ventilation to be increased beyond the level required by the metabolism of the body. This reflex increase in respiration furnishes the physiologic basis for many of the symptoms of the psychoneurotic patient. The patient may be conscious of the hyperventilation itself and complain primarily of dyspnea, or he may complain of any of the resultant symptoms, not being aware of the increased pulmonary ventilation.

2. Voluntary hyperventilation in normal subjects produces a disturbance in cerebral metabolism with variable symptoms. Usually faintness, giddiness or some similar complaint is followed by numbness and tingling about the mouth and extremities; the hands become cold, and if the patient is standing he may faint. Prolonged hyperventilation may produce signs and symptoms of tetany.

3. Any of the cerebral symptoms produced by voluntary hyperventilation may appear in the anxious patient who unknowingly hyperventilates. Production of these symptoms by voluntary overbreathing is not only of diagnostic aid, but is useful in demonstrating to the patient that his symptoms have a physiologic rather than a pathologic basis.

4. At times the hyperventilation itself may be noted by the patient and may appear as a symptom, particularly in patients with cardiac disease without congestive failure or in patients who fear cardiac disease. Associated with this breathlessness there may be other symptoms of hyperventilation.

5. Observation of the effects of voluntary hyperventilation should be a routine procedure in the examination of: (1) patients complaining of fainting, giddiness, or a far-away feeling; and (2) patients with breathlessness, particularly those with cardiac disease without evidence of congestive failure.

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A MODIFIED CHRISTIE METHOD FOR RESIDUAL AIR MEASUREMENTS

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A method for the measurements of the functional residual air, based on the dilution of the nitrogen in the lung was described by Christie[4] in 1932 (review of literature). A spirometer filled with oxygen was attached to the test subject. The carbon dioxide was removed with soda lime, and the subject rebreathed in the circuit until nitrogen equilibrium was reached throughout the lung-spirometer system. The functional residual air was calculated from the composition of the gas in the spirometer. It was assumed that after 5 or 7 minutes of quiet breathing, gas equilibrium was complete in lungs and spirometer, except for a slightly greater concentration of nitrogen in the lungs. This excess was assumed to be the same as that occurring during the breathing of room air. A correction for the nitrogen excretion in the lungs was made.

Lassen, Cournand and Richards[13] found a lower concentration of nitrogen in the lungs than in the spirometer at the end of the rebreathing period. They called this effect "oxygen storage" and attributed it to the decreasing volume of gas in the spirometer due to oxygen consumption. They corrected the oxygen storage effect by introducing the alveolar nitrogen concentration in the calculation of the residual air.

Herrald and McMichael[10] proposed to keep the spirometer volume constant with a continuous oxygen flow during the rebreathing period, in order to avoid the oxygen storage effect.

Anthony,[1] using the hydrogen dilution method, found no storage effect, provided that the spirometer volume was at least 3 times larger than the expected residual air. It should be noted that he used a spirometer equipped with a powerful blower.

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Keltreider *et al.*[11] found that the alveolar percentage of nitrogen was higher than in the spirometer. However, the difference was less than than between alveolar nitrogen, before the rebreathing period, and atmospheric air.

Cournand *et al.*[5] presented the results of functional residual air measurements using the original Christie method, the Lassen *et al.* modification, the constant volume technique of Herrald and McMichael and the increasing lung nitrogen method. The results gave agreement by all methods in 4 normal subjects only, 3 of whom had small residual air volume. In the 2 other normal subjects there were wide discrepancies between the results with different methods. Keeping the spirometer volume constant did not correct the discrepancies. They consider the data presented as a positive evidence of unequal gas distribution within the lungs.

Darling *et al.*[8] and Cournand *et al.*[6] used a new open circuit method to avoid the inadequacy of the closed circuit method. The reasons of such inadequacy would be: (1) the changing composition of inspiratory gas mixture, due to reduction of the spirometer gas volume as oxygen is absorbed; (2) difficulties in the exact calculation of the oxygen absorbed in some subjects; (3) magnification of any error in the final residual air value due to the small net change in lung nitrogen concentration, usually no more than 0.3 of an atmosphere.

In the open circuit method the subject is allowed to breathe oxygen for a period of time sufficient to wash practically all the nitrogen out of the lungs. For this period all the expired gases are collected, measured, and analyzed. Alveolar samples are taken before and at the end of the test. Cournand *et al.*,[7] from 158 determinations of the lung nitrogen concentration during room air breathing, concluded that an assumed value of 81% of nitrogen in the lungs before the rebreathing period can be used without introducing a significant error in the final calculation, thus avoiding one of the alveolar samples.

Meneely and Kaltreider[15] found that with the original Christie method the average values of residual air were 292 cc., and with the Lassen *et al.* modification, 202 cc. higher than with the helium method.

Another factor which introduces errors in the residual air measurements is the nitrogen excreted in the lungs during the rebreathing period. Darling *et al.*[9] found that the nitrogen excreted in 7 minutes of breathing pure oxygen averaged 220 cc. in 4 normal subjects, figures which are similar to those obtained by Campbell and Hill.[2]

Cournand, Yarmush and Riley[7] studied the nitrogen excretion on 30 subjects, during 7 minutes of oxygen breathing. From it they calculated a regression formula for nitrogen excretion.

From this brief review it is clear that the modification introduced into the Christie method makes it more laborious, without correcting completely the discrepancies with the other methods.

The open circuit method, being from a theoretical point of view the best, offers in practice some disadvantages, such as the lack of a graphic record of the expiratory level, and the necessity for extreme accuracy in the nitrogen analysis.

We have endeavored to combine in the Christie procedure the advantage of a large change in the net nitrogen concentration of the alveolar air with that of having a graphic record of the starting expiratory level of the subject, thus making it an exact and simpler procedure for routine measurements.

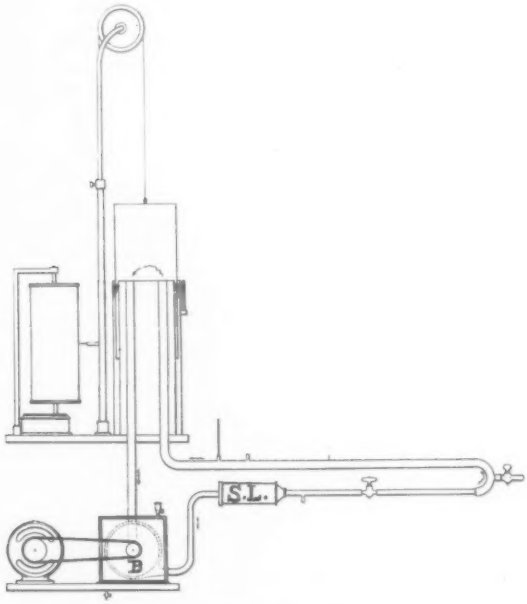

FIG. 1.—Modified Knipping apparatus. *B*, Blower; *S.L.*, soda lime container.

Procedure. The apparatus[3] used was a modified Knipping[12] spirometer of 5 liters capacity (Fig. 1). The blower was immersed in an oil bath for gas tightness. The connecting tubes were metallic, and on the inspiratory side there was a soda lime container for carbon dioxide absorption. A double set of 3-way stopcocks permitted alveolar air sampling, before and at the end of the rebreathing period. The dead space of the spirometer was 2865 cc., which made the total capacity of the circuit about 8 liters.

The procedure for a determination of functional residual air was as follows: With the blower motor on, the circuit dead space was washed out 10 times with pure oxygen (nitrogen impurities must be known). Oxygen was then added, usually about 5000 cc. to fill the spirometer bell. The subject, under basal conditions, was made to breathe through a mouthpiece, and an alveolar sample was taken. After 2 or 3 minutes the mouthpiece was connected to the spirometer at the end of a normal expiration and a 7 minutes period of rebreathing was allowed; at the end of it, another alveolar sample was taken.

The spirometer and alveolar samples were analyzed in a Shepherd apparatus. Duplicates checked within 0.10%. Carbon dioxide content of alveolar specimens was used to detect dilution with dead space gas.

All volume measurements were made from the tracings. The volume of added oxygen was recorded at the time it was run in, the oxygen consumption was measured from the slope of the tracing. The initial volume minus oxygen consumption equals the final spirometer volume to which the dead space volume was added. If needed, temperature corrections were made.

Calculations were made following the original Christie formula (1) and the Lassen *et al.* modification (2).

$$\text{F.R.A.} = \frac{\bar{V}p(\text{Spir.p}) - \bar{V}a(\text{Spir.}\bar{a}) - N_2 \text{ excretion}}{0.791 - \text{Spir.p}} \quad (1)$$

$$\text{F.R.A.} = \frac{\bar{V}p(\text{Spir.p}) - \bar{V}a(\text{Spir.}\bar{a}) - N_2 \text{ excretion}}{\text{alv.}\bar{a} - \text{alv.p}} \quad (2)$$

F.R.A. = functional residual air in cc.
$\bar{V}p$ = final spirometer volume plus dead space.
$\bar{V}a$ = initial spirometer volume plus dead space.
Spir.p = final spirometer nitrogen expressed as part of an atmosphere.
Spir.$\bar{a}$ = initial spirometer nitrogen expressed in same units (due to impurities in oxygen).
Alv.$\bar{a}$, Alv.p = alveolar nitrogen content in same units.

Nitrogen excretion was calculated by the regression formula given by Cournand *et al.* for 7 minutes of pure oxygen breathing.

Nitrogen excretion (cc.) = 96.5 body surf. area (sq.m.) + 35

It was assumed that the nitrogen excretion is proportional to the fall of the gas pressure and that 0.8 of an atmosphere is the change occurring after 7 minutes of oxygen breathing (Darling *et al.*[9]).

The volume of the mouthpiece dead space, 20 cc., was subtracted from the final result. Functional residual air thus obtained may need a correction if the rebreathing period did not begin at the exact end of a normal expiration. Such a correction was determined from examination of the tracing.

Duplicate measurements were made the same morning with a 45 minutes' interval.

Results were corrected to 37° C., and saturation with water vapor.

When measurements with original Christie procedure were made, the apparatus was washed out with room air, and the spirometer bell only was filled with pure oxygen.

McMichael technique was used for hydrogen measurements. The gas samples were analyzed by duplicates in a Shepherd combustion apparatus with an accuracy of 0.10 cc. %.

Results. Subjects were 3 normal males and 14 females with different types of pulmonary lesions without emphysema. Physical characteristics of the subjects are presented in Table 1.

TABLE 1.—PHYSICAL CHARACTERISTICS OF PATIENTS AND NORMAL SUBJECTS STUDIED

Patient	Sex	Age (yrs.)	Stature (m.)	Body surf. area (sq. m.)
A. B.	M	42	1.795	2.09
R. C.	M	23	1.81	1.94
A. F.	M	47	1.68	1.87
A. S.	F	48	1.50	1.42
D. S.	F	39	1.58	1.67
I. G.	F	28	1.52	1.28
M. B.	F	43	1.525	1.26
M. S.	F	39	1.55	1.62
C. S.	F	16	1.62	1.60
C. P.	F	24	1.56	1.59
D. M.	F	28	1.545	1.48
C. S.	F	16	1.62	1.60
L. R.	F	30	1.635	1.77
C. C.	F	28	1.50	1.54
D. F.	F	22	1.575	1.58
R. G.	F	28	1.575	1.43
M. G.	F	35	1.52	1.56

Tables 2 and 3 present in some detail our experimental results. The difference between figures in Columns 3 and 4, giving a measure of the oxygen storage effect are small and negligible in all cases by the authors' technique. It is probably from the trends in our data that with larger volumes of residual air this effect would become significant; it is likewise probable that in the original Christie technique the effect would appear with smaller residual air volumes.

TABLE 2.—FUNCTIONAL RESIDUAL AIR OF PULMONARY TUBERCULOUS PATIENTS, WITHOUT EMPHYSEMA

Subject	Column: Date 1942	1 Alveolar nitrogen at the end (%)	2 Spirometer nitrogen at the end (%)	3 Christie calculation (cc.)	4 Lassen *et al.* calculation (cc.)	5 Hydrogen method (cc.)	6 3 minus 4 in % of 4 (%)	7 Difference between duplicates† (%)	8 Maximal difference on same subject† (%)
A. S.	3/13 M	16.90	15.41	1342	...	...	...	0.8	
	3/18	17.16	15.34	1353	...	...	...	...	1.2
D. S.	3/16	18.35	17.36	1459					
	3/17	17.37	15.83	1353	...	...	...	...	7.8
	3/20	17.57	16.48	1370					
I. G.	3/10 M	17.34	15.11	1273	...	...	...	3.4	4.9
	3/11	16.74	15.15	1235					
M. B.	3/5	...	14.06	1096					
	3/16	15.86	14.06	1078	...	...	...	...	3.5
	3/25 M	13.16	13.03	1106	1073	...	−2.7	1.5	
M. S.	3/30	11.30	10.81	742	731	...	−1.5		
	1/4 M	16.94	10.62	705	698	...	−1.0	1.0	5.7
C. S.	4/15 M	16.56	15.65	1204	1191	...	−1.7	1.1	5.1
	4/17 M	15.85	14.63	1156	1121	...	−3.1	0.8	
C. P.	4/27 M	15.53	14.93	1146	1109	...	−3.4	7.5	
	4/29	16.72	15.32	1168	1130	...	−3.4	...	8.2
	5/6 M	*48.49*	*47.01*	*1411*	*1392*				
	5/8	*40.13*	*44.55*	*1404*	*1385*				
	5/8	15.18	14.88	1098	1068	...	−2.8		
D. M.	4/22 M	19.27	18.38	1435	1436	...	−0.5	2.1	
	4/24	18.53	16.76	1284	1316	...	−2.4	...	14.8
	5/4	18.89	17.30	1459	1457	...	−0.1		
	5/4	*49.47*	*47.35*	*1836*	*1864*				
C. S.	5/29	...	14.14*	...	...	919			
	5/29	13.94	12.85	903	915	...	+1.3		
	6/1	14.38	12.98	956	949	...	−0.7		
	6/1	*45.28*	*44.41*	*1173*	*1140*				
L. D.	6/8 M	18.17	16.15	1303	1303	...	−0.8	1.9	
	6/9 M	...	12.41*	...	...	1234			
	6/10 M	*49.08*	*46.78*	*1530*	*1503*				
C. C.	6/15 M	21.45	18.88	1816	1625	...	+1.1	0.7	
	6/16	...	13.28*	...	...	1398	...	...	7.5
	6/17	...	13.46*	...	...	1619			
	6/17	...	18.28	1631					
D. F.	7/4 M	...	12.93*	...	...	1133			
	7/7 M	17.48	14.32	1191	1202	...	+2.2	2.6	
	7/8 M	*46.10*	*45.34*	*1498*	*1485*				
R. G.	7/13 M	...	13.96*	...	...	1117			
	7/14 M	15.40	14.48	1254	1248	...	−1.6	2.3	
	7/15 M	15.01	14.57	1199	1184	...	−1.2	2.8	
M. G.	7/17 M	...	18.24	1549	...	...	...	6.7	
	7/20 M	16.93	15.69	1338	1348	...	+0.8	0.2	20.0
	7/21 M	18.49	15.77	1364	1423	...	+4.2	5.3	

Figures in italics were obtained with the original Christie method.
* Spirometer hydrogen percentage at the end.
† Calculated from Column 3 values in percentage of the lowest value. Only author's technique results have been included.
M, mean values of duplicate determinations listed for these dates.

TABLE 3.—FUNCTIONAL RESIDUAL AIR OF NORMAL SUBJECTS

Subject	Column: Date 1942	1 Alveolar nitrogen at the end (%)	2 Spirometer nitrogen at the end (%)	3 Christie calculation (cc.)	4 Lassen *et al.* calculation (cc.)	5 Hydrogen method (cc.)	6 3 minus 4 in % of 2 (%)	7 Difference between duplicates† (%)	8 Maximal difference on same subject† (%)
A. B.	5/18	21.14	21.08	2047	1998	...	−2.5	...	5.1
	5/20	*50.42*	*50.58*	*2502*	*2289*				
	5/21	21.91	21.28	1947	1923	...	−1.2		
	5/21	...	16.21*	...	...	1928			
R. C.	5/22 M	25.44	24.96	2430	2416	...	−0.6	2.5	
	5/27 M	...	15.06*	...	...	2244			
	5/28 M	*51.21*	*51.49*	*2835*	*2707*				
A. F.	6/18 M	23.08	22.39	2142	2047	...	−4.6	5.1	
	6/19	...	13.39*	...	...	2294			
	6/19	...	14.59*	...	...	4176			
	6/22 M	*50.84*	*51.00*	*1765*	*1695*				

Figures in italics were obtained with the original Christie method.
* Spirometer hydrogen percentage at the end.
† Calculated from Column 3 values in percentage of the lowest value. Only author's technique results have been included.
M, mean values of duplicate determinations listed for these dates.

Nitrogen fall in the alveolar air ranged from 0.55 to 0.69 of an atmosphere with the author's procedure, and from 0.28 to 0.33 with the original Christie method.

Hydrogen concentration was kept between 13% and 15%.

Among both normal subjects and tuberculous patients, duplicate determinations made the same day and on the same subject check within 2.5% as an average. The checks range from 0.7% to 7.5%. On the other hand, measurements made on different days on the same subject show a maximum difference of from 1.2% to 20% (average 7.9%).

Duplicate measurements by the hydrogen method or by the original Christie method show wider ranges in most cases.

By the hydrogen method, values average 56 cc. lower than our own values.

Using the original Christie technique on 8 subjects, results are, on an average, 215 cc. higher than those calculated with Lassen *et al.* formula, and the difference ranges from +3.8% to −5.5%. This difference is rather constant when measurements on the same subject are repeated.

Discussion. From our results it is apparent that, when a large net change in the alveolar nitrogen percentage is produced, and a rebreathing apparatus equipped with a powerful blower is used, the oxygen storage effect as described by Lassen *et al.*[13] is not present. Such a lack of a storage effect can be explained by the fact that the changes of nitrogen percentage in the inspired gas, produced by oxygen consumption, are lessened by the large volume of the circuit, the low nitrogen concentration, and by the rapid mixing of the gases in the spirometer.

The total volume of the spirometer circuit at the end of the experiment must be at least 3 times larger than the expected residual air, a fact which was already pointed out by Anthony[1] and confirmed by our own measurements.

But even in cases where no oxygen storage effect is present, the determination of residual air volume by using small net changes in alveolar nitrogen content gives higher values than when larger nitrogen changes are used as in the author's technique.

Likewise, Meneely and Kaltreider[15] found higher values with the original Christie method, and Lassen *et al.* modification, than those obtained with the helium method. Furthermore, the results presented in this paper using the hydrogen method agree with those of the author's modified Christie method.

It is then evident that the use of a large net change in the alveolar nitrogen concentration corrects the errors of the original Christie method, errors which the introduction of the alveolar nitrogen percentage in the calculations[13] or the constant volume technique[10] did not remove.

It cannot be decided whether the more correct results are due only to a better mixture of lung gases induced by great differences of the nitrogen pressure (at the start) between alveoli and spirometer or if the final low nitrogen percentage, not magnifying the errors in the calculations and giving a greater sensitiveness to the method, is also an important factor.

That a greater sensitiveness exists is shown by the fact that, using our method, a given change in the volume of the measured residual air induces in the final nitrogen percentage a change 100% greater than using the original Christie method.

The nitrogen excreted by the lungs during the rebreathing period introduces another cause of error in the residual air measurements. The errors by miscalculation of the excreted nitrogen are more conspicuous and significant the smaller the residual air values, and the use of a single correction standard in all subjects, as that obtained from the Cournand *et al.*[7] regression formula, may seem an unjustified assumption. We made also the assumption that the nitrogen excreted would be proportional to the fall in the lung nitrogen pressure.

However, the agreement of our values, even in cases with very small residual air, with those obtained by the hydrogen method, in which the nitrogen is kept at atmospheric pressure during the whole rebreathing period, seems to justify the use of the Cournand *et al.*[7] regression formula. On the other hand, errors by miscalculation of the hydrogen dissolved in the blood are very small.

Only slight differences exist between results calculated by the original Christie formula and by the Lassen *et al.* modification. It appears then that using our technique the original Christie formula may be used without introducing significant errors in the residual air values. Alveolar air samples might then be omitted, and a real simplification could be obtained.

In conclusion, the advantages of the proposed technique for residual air measurements, as they must be done on pulmonary tuberculous patients, are clear-cut. By increasing the net alveolar nitrogen change it offers a better chance of adequate intrapulmonary gas mixing, and it does not magnify the errors in the final result. By avoiding alveolar sampling and extremely low final nitrogen percentage in the spirom-

eter, which would demand very accurate analysis, it simplifies the measurements without introducing significant errors in the results. Finally, the functional residual air values can be corrected for differences in the expiratory level at the start, from the examination of the graphic. Such a correction is particularly useful in cases of nervous patients.

Summary. A modification of the Christie method which makes it suitable for routine examinations, is described. Errors are minimized by increasing the change in concentration of the alveolar nitrogen. Alveolar sampling is avoided, analysis need not be so accurate. A graphic record of the expiratory level is obtained. Results on 14 tuberculous patients without emphysema and 3 normal subjects were compared with those obtained by the original Christie method and the McMichael hydrogen method. The significance of the results is discussed.

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DIABETES AND THE WEATHER

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IN a recent paper Beardwood and Rouse,[1] studying the onset of diabetic coma in 220 cases, found that in 100 of them no obvious cause was to be ascertained. In a later paper, Joslin, Root, White and Marble[3] came to the conclusion that the "commonest cause of diabetic coma

continues to be laxity in treatment consisting either of dietary indiscretions or neglect to take an adequate amount of insulin, or both."

This point is driven home with the clinical demonstration that with prompt and proper dosage of insulin, fluid, and salts, the coma can be overcome.

The situation, however, is not as simple as might therefrom be inferred. Every physician who has followed his cases carefully and whose patients have followed routine instructions faithfully is confronted, as Beardwood and Rouse make evident, with the sudden precipitation of coma, and both physician and patient cannot adequately explain the reason.

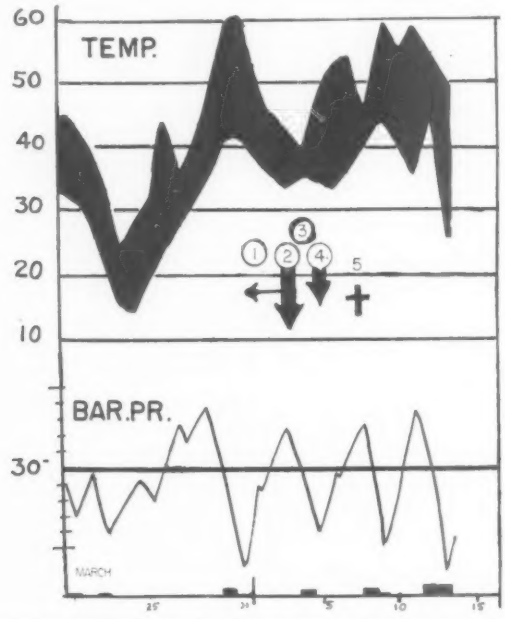

FIG. 1.—Meteorogram to illustrate the clinical events in Case 1 of Drs. Joslin, Root, White and Marble.

Before proceeding to the rather involved discussion, I shall examine 3 case reports detailed by Dr. Joslin and his associates, to illustrate a very simple association of the condition of the patient to the environmental situation which may play a rôle in aggravating the picture.

Joslin's Case 1 was moribund when first seen on April 3, 1940 (episode [2] in Fig. 1). The history indicated that he had eaten little or nothing for 3 days previously (1). After improvement the patient again became drowsy (3, 4); then improved, but died suddenly on the 8th of April (5). When we examine the meteorogram (Fig. 1) for the period under consideration we observe the following: Environmental temperatures had been relatively high at the end of March, barometric pressures had been low and with the passage of this cyclonic

air mass, rain had fallen. Then followed a polar episode—temperatures declined to 40° F., a barometric crest obtained when the patient was first seen. Sudden death occurred with the next barometric crest. We turn to the next 2 cases (Fig. 2) and immediately note that these 2 patients died at approximately the same time (August 24 and 25). Such coincidence is accepted as chance. But is it? According to the record, Patient No. 2 was admitted on August 13 and Patient No. 3 on August 17, both in acidosis, although the exact time of the onset was not indicated in the record.

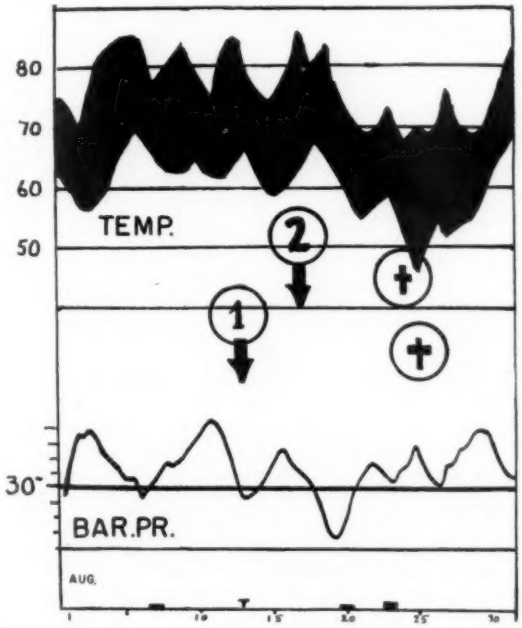

FIG. 2.—Meteorogram to illustrate the clinical events in Cases 2 and 3 of Drs. Joslin, Root, White and Marble.

When we now examine this meteorogram we note that death occurred when temperatures had fallen from maxima in the eighties to lows in the forties and air pressure had increased to a crest.

Do such environmental situations alter the metabolism of the healthy or diabetic individual and do they play a rôle in precipitating acidosis?

The Exceptional Requirement of Insulin and Salt Solution in Diabetic Coma. In Root and Riseman's[7] interesting study of the use of insulin and salt solution in diabetic coma, 2 case reports have been dated: The first concerned a woman, 31 years of age, who was admitted to the Beth Israel Hospital in Boston on April 22, 1937 (episode [3] in Fig. 3). Four days previously she had lost her appetite (1), and 2 days previously she had been short of breath and vomited (2). On the morning of admission she was found unconscious.

Examination of the meteorogram (Fig. 3) immediately indicates the onset of the disturbance when temperatures had fallen from a maximum of 70° to 35° and an increase in the severity of the symptoms (hospitalization) occurred with the succeeding barometric crest and fall in temperature.

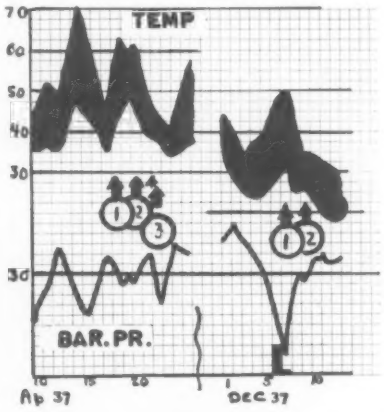

FIG. 3.—Meteorogram to illustrate the relation of coma and the clinical course in Cases 1 and 2 in Root and Riseman's report.

In the second case described, a teacher, 25 years of age, who was a diabetic, had been taking 50 units of protamine zinc insulin to September 4. On December 7 she felt as if she were getting a mild attack of grippe (episode [1] of second case in Fig. 3) and from 10 o'clock on she vomited every 10 minutes. She was admitted to the hospital at 5 P.M. on December 8 quite unconscious (2.)

Examination of the meteorogram immediately indicates the association of the entire clinical episode with the passage of a sharp polar front, 3 in. of precipitation having occurred on the 6th of the month, more than $\frac{1}{2}$ in. on the 7th and barometric pressures increased more than 1 in. in 2 days' time!

Organic Variability. For the complacent acceptance of a static rather than a dynamic concept of the human organism (the interested reader might turn to Lawrence Henderson's[2] discussion of this subject), Cannon's term "homeostasis" provided an assurance that was apparently very satisfying. There can be no question of its general validity, but within this framework there appears a wide latitude of chemical movement and when we study the biochemistry or the physiologic function of the normal or sick human we find that the organism is never the same from day to day.

Every passing phase of sympathicotonia, with its increase in diastolic and systolic blood pressure, is associated with a transient anoxia (largely peripheral), this involves the production of more acid products because of the anoxybiosis. Tissue stimulation follows, and is reflected in the capillary bed with an increase in permeability, and this, in turn, is associated with greater tissue hydration. But every passing phase of sympathicotonia is also associated with sugar mobilization

from the liver stores of glycogen and this sugar is released at a time when the peripheral bed (skin, muscles, connective tissue) is relatively constricted and the mobilized sugar cannot be delivered to the areas where most of it would be utilized under normal conditions, provided adequate insulin were available.

Any sympathicotonia therefore tends to accentuate the diabetic state; when repeated in rapid sequence or when amplified because of violent environmental changes, a background is laid for diabetic coma.

Cold, relative or actual; hyperventilation; undue excitement; any infection or trauma; any sudden disturbance of metabolic equilibrium (Hippocrates) throws a greater burden on mechanisms of autonomic equilibration, and in so doing precipitates a diabetic crisis.

Obviously insulin dosage must be such that it provides a wide margin of safety for these constant pendulations and amplifications of the organic tide. When the margin of adequacy is restricted, a seemingly minor change in the environmental condition may be followed by disaster. It is for this reason that the commonplace changes in the environment associated with weather and season must be seriously drawn into the sphere of interest of the attending physician and the patient.

To illustrate this I turn to a simple example from the records of the Children's Memorial Hospital, generously placed at my disposal by Dr. Newcomb.

The cases admitted in coma for the year 1932 are indicated in Table 1 and it will be observed that 3 young children were admitted in coma at the same time (February 2 and 4). Why the sudden precipitation of an acute condition?

TABLE 1.—ONSET AND HOSPITAL ADMISSION DATES OF DIABETIC CHILDREN (1932)
(Dr. Newcomb)

Name	Sex	Dur. of disease on admission (wks.)	Age at onset	Date of onset	Admission
S. O.	F	3	2–10	1–13–32	**2– 2–32**
D. S.	M	2	2– 4	1–21–32	**2– 4–32**
D. O.	M	1	2– 3	1–27–32	**2– 2–32**
D. K.	F	8	8– 5	2–21–32	4–21–32
B. B.	F	16	11– 9	5– 2–32	8–16–32
D. N.	M	8	2– 0	5– 5–32	7– 5–32
F. W.	M	2	1– 3	7–14–32	7–28–32
K. C.	M	20	8– 3	7–20–32	12–20–32
A. L.	M	2	1– 2	9–10–32	9–24–32
R. R.	F	3	2–10	10– 6–32	10–27–32
G. H.	M	3	8– 0	10–15–32	11– 8–32
E. S.	M	3	11–11	10–21–32	11–12–32
E. B.	M	2	8– 9	12–15–32	12–29–32

We turn to examine the meteorologic situation at the time as illustrated in Figure 4. It will be observed that temperatures declined from 50° F. to 0° on January 31. Then temperatures rose abruptly. With such a situation a relative anoxia can readily be demonstrated in the normal as well as in the sick individual. The peripheral blood bed tends to close, sugar is mobilized, it is kept from the periphery at the same time that acid metabolites accumulate. After this phase, vessels become more permeable, tissues swell, in the potential or actual diabetic coma can develop. (Arrows A and B, February 2 and 4.)

When we examine the day by day records of diabetic deaths for Chicago at the time when this episode occurred in children we can observe a characteristic crest in the number of deaths. In other words, the sudden precipitation of diabetic coma in these young children was not a chance phenomenon, others in the diabetic population were also involved.

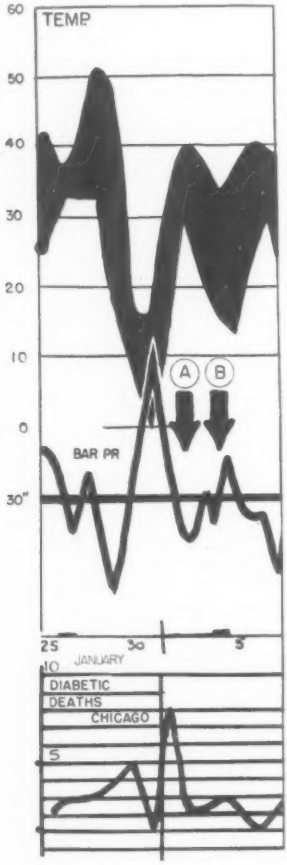

FIG. 4.—Meteorogram to illustrate death episodes discussed in Dr. Newcomb's Table of Admissions of Diabetic Children. Upper curve, maximal and minimal temperatures; heavy solid curve, barometric pressures; lowest curve, daily diabetic deaths. Chicago records for the period.

Variability of Sugar Output in the Diabetic. Among the curious phenomena of diabetes that interested Naunyn, the founder of our modern knowledge of diabetes, was the obvious fluctuation in the sugar output in the diabetic who was kept on an absolutely controlled sugar régime. Naunyn[4] could not account for it. I shall illustrate his findings by introducing one of his own controlled observations, using his original chart. (Fig. 5.)

To make the association of decrease in environmental temperatures and increase in sugar output readily evident I have merely numbered a few of the outstanding periods (circled numbers 1 to 10) on both curves.

During August, two major polar air masses swept over Strassburg (1) and (2). The increase in barometric pressure and fall in temperature is readily apparent. To this change the two sugar crests (1) and (2) are related.

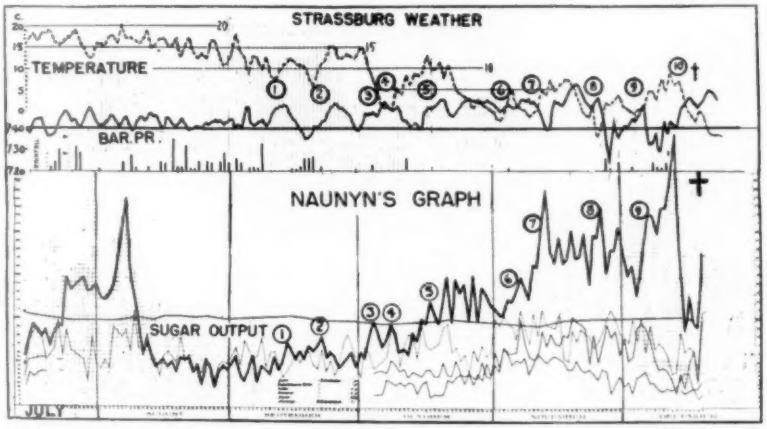

FIG. 5.—Naunyn curve of sugar output with superimposed meteorogram for Strassburg. Naunyn's curve of sugar excretion in a patient observed in Strassburg from July 14 to his death on December 19. Controlled regimen became effective about August 10. Increased excretion occurred in short waves of approximately 4 to 5 days' duration. Major increase began early in October. Dotted line, mean daily temperature in centigrade; solid line, barometric pressure in millimeters; black columns, precipitation.

Early in October (3) and (4) an increase in sugar output is again apparent. Environmental temperatures fell from 15° C. to 0°. With this the sugar curve rose sharply and on October 16th barometric pressures again rose abruptly—a sugar crest was evident on the 17th (5). A temperature decline then followed from the 17th for the balance of the month and sugar levels remained high. A crest on November 3d followed episode (6).

In this fashion each major environmental change finds its reflection in change in the sugar level reflecting a mobilization and a distribution and the accumulation of sugar as the result of defect in utilization. In this case the final sugar crest occurred on December 13th, following an increase in barometric pressure from the lows of December 10th and 11th. The patient then died with the further development of the cold wave.

Discussion and Summary. The interrelation of environmental temperature and air pressure and the underlying biochemical changes that occur in the human have been discussed in detail in a recent publication (5), and the diabetic problem has been discussed fully elsewhere.[6]

The clinical inference might seem obvious, namely, fortifying the patient by increasing the insulin intake with changing seasonal and weather demands. The intelligent patient can be instructed to modify the dosage with change in weather. Possibly even more important is the recognition of the hazard involved in adjustment to cold and the consequent instruction of the patient to promptly make use of adequate clothing, suitable room temperatures, the avoidance of unnecessary exposure and taxing environmental situations of whatever nature. The same physiologic adjustment that occurs with cold is basically involved in fear and anger or excitement, in the early stages of infection, in gastro-intestinal overload, in allergic reactions, in pain, and finally in fatigue.

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PRECLINICAL GENITO-URINARY TUBERCULOSIS

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Ricord's classification of the luetic infections into primary, secondary and tertiary stages was so striking and fundamental that it probably influenced Ranke to apply similar categories to tuberculosis (1916). To substantiate this thesis Ranke could point to Naegeli's postmortem findings (1900), v. Pirquet's doctrine of allergy (1906), and Ghon's study of the character of the primary infection (1912). Since then phthisiologists are agreed that in tuberculosis there are 3 stages of development: the analogy with syphilis is basically correct. However, chronologically, the succession of the stages is not always I, II, III, in a forward progression as Ranke taught. Stage I can become III without an intermediate hematogenous dissemination (II), and an isolated organ tuberculosis (III) can give rise to recurring or a single episode of II. Ranke's classification restricts the evolutionary possibilities in a given individual. Tuberculosis is more dynamic

TABLE 1.—CLINICAL SUMMARY OF CASES

Case	History	Pulmonary status, other diagnoses	Urine	PSP (%)	Conc.	Urologist's impression
M. 49 Japanese Male	6 mos.; pleurisy, effusion, gonorrhea	IIA, sputum pos.; pleurisy with effusion, bilateral	Alb. 2+ Incr. WBC RBC Pos. cult.: 4 of 7	92	1006–30	Tubere. prostatitis and epididymitis; calcific. in right kidney; tubere. of left kidney
W. 28 White Female	6 mos.; pleurisy, effusion	No parenchymal disease; resolved pleural effusion, right	Alb. tr. to 1+ Pos. cult.: 1 of 3	..	1014–25	Negative
M. 34 Philippine Male	10 mos.	IIC, sputum pos.; left pleural effusion	Alb. 4+ Incr. WBC Hematuria Pos. cult.: 2 of 2	50	1006–24	Tubere. of left kidney; ulceration of bladder with perforation
N. 23 Negro Male	2 yrs.	IIA, sputum pos.; right hydropneumothorax	Alb. tr. RBC Pos. cult.: 1 of 7	..	1015–20	Negative
T. 25 White Male	1 yr.	IA, sputum neg.; bilateral pleural thickening	Alb. tr. to 2+ Incr. WBC Pos. cult.: 2 of 2	80	1005–15	Cystoscopy: neg.; intraven.pyelography: dis. of left kidney; postop. specimen; caseous-ulcerative tubere. left kidney
R. 43 Negro Male	3 yrs.	IIIB, sputum pos.; hematogenous infiltration of the lungs; brain tuberculoma (?); Pott's disease	Alb. 2+ Incr. WBC RBC Pos. cult.: 3 of 3	96	1013–20	Cystoscopy: granulation tissue throughout lower half of bladder, ureters contracted, questionable patency on right; retrograde: left kidney disease
H. 21 Negro Male	4 mos.	IB, sputum pos.; bronchial lymph node penetrating into bronchus; tuberculosis of both knees; Pott's disease	Alb. 2+ Incr. WBC Pos. cult.: 2 of 6	89	1026–33	Epididymo-orchitis left; postop. specimen: same; cystoscopy: neg.; pyelography: right kidney disease
N. 24 White Male	13 mos.	IIB, sputum pos.	Alb. tr. Pos. cult.: 1 of 1	..	1014–22	Negative
R. 22 White Female	13 mos.	IA, sputum neg.; bilateral pleural thickening	Alb. tr. Pos. cult.: 1 of 6	..	1010–25	Negative
M 43 Negro Male	10 mos.	IA, sputum neg.	Alb. 2+ Incr. WBC RBC Pos. cult.: 2 of 5	88	1008–27	Negative
V. 29 White Male	3 wks.; pleurisy, effusion, right	No parenchymal disease; thickened pleura, right	Alb. tr. Incr. WBC Pos. cult.: 5 of 5	96	1014–22	Cystoscopy: granulation tissue and ulceration at both ureteral openings, more so at right; pyelography: left kidney disease; postoper. specimen: caseous tubere. of left kidney and ureter
K. 19 White Male	3 mos.	IIA, sputum pos.	Alb. tr. Incr. WBC Pos. cult.: 0 of 3	..	1010–26	Epididymitis, right
M. 24 White Male	7 yrs. "pleurisy"	IIB, sputum pos.	Alb. tr. Incr. WBC Pos. cult.: 2 of 4	58	1014–27	Cystoscopy: scarring and constriction of left ureteral opening; intraven. pyelography: disease of left kidney
M. 19 White Male	5 mos.	IIB, sputum pos.; hematogen. infiltration in lungs; calcification in mesenteric lymph nodes	Incr. WBC Pos. cult.: 3 of 3	43	1005–23	Cystoscopy: neg.; pyelography: left kidney disease; postop. specimen: caseous ulcerative tuberculosis
B. 17 Colored Male	5 mos.	Penetrating bronchial lym. node, terminating in miliary dissemination and penetration into heart	Alb. 2+ Incr. WBC Pos. cult.: 2 of 3	..	1024–26	Negative; postmortem: miliary tubercles throughout all organs, Pott's abscess, caseous tuberculosis of heart

in its course than is pictured in his straight line sequence of stages. Figure 1 would be more characteristic of the changes that can occur.

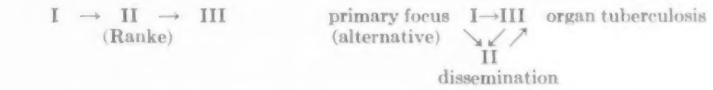

FIG. 1.—Stages occurring in tuberculosis, Ranke and alternative sequences.

With this scheme in mind, it will be realized that tuberculous disseminations can be a frequent possibility. Consequently, the eagerness to diagnose extra-pulmonary foci should be as keen as our endeavors in any pulmonary survey. The urine offers a ready and adequate source for the investigation of genito-urinary tuberculosis, and here the laboratory can ever be the first to report an excretory tuberculosis. A normal urine in the female contains up to about 12 WBC per high-power field per centrifuged specimen of 15 cc., in the male up to about 6. In a tuberculous individual any increase in the number of the pus cells or the presence of red blood cells or albumin should automatically call for a 24-hour specimen to be concentrated for tubercle bacilli. The smear from such concentration allows only a preliminary report. As a routine, the residue should then be treated and cultured on a standard medium for tubercle bacilli. Such concentrates and cultures of 24-hour specimens are to be repeated monthly or bi-weekly until a sufficient number of reports are available for the urologist to plan his cystoscopic and retrograde examinations.

This is the routine employed at the Municipal Sanatorium. Our institution has been receiving minimal and moderately advanced cases* of pulmonary tuberculosis from the clinics and hospitals of the City of New York. Since the induction of our urine routine, we have uncovered a relatively high percentage of excretory tuberculosis. The high figure occurring in a sanatorium population with comparatively little chest disease emphasizes the necessity of being on the alert to diagnose latent or preclinical genito-urinary tuberculosis.

Data. During August, 1940 to February, 1942, 393 24-hour urine specimens were studied for tubercle bacilli because routine specimens from the same patients revealed contents deviating from the absolute normal. These came from 214 different individuals. Tubercle bacilli were isolated in 29 instances, representing 14 individual cases. One individual had definite clinical evidence of a tuberculous orchitis, yet urine specimens were persistently negative for tubercle bacilli. Thus, of 214 suggestive cases, 15, or 7% yielded positive clinico-pathologic findings (Table 1). The positive cases comprised 2 yellow males, 5 negro males, 6 white males, and 2 white females. Their ages varied from 17 to 49. Of these individuals, 3 gave a definite history of pleurisy with effusion, 1 claimed to have had "pleurisy," 1 developed a pleurisy with effusion under observation, and 2 showed evidence of thickened pleuræ roentgenographically or radiographically. Three cases had

* In 1941, 557 admissions, of which 51.4% were classified as minimal, 41.6% moderately advanced, and 3.4% far advanced. The remainder were non-tuberculous.

chest films characteristic of a hematogenous dissemination through the pulmonary circuit, and 4 had simultaneous bone lesions (3 Pott's, 1 knee). Table 2 gives the distribution of the genito-urinary pathology in our group.

TABLE 2.—GENITO-URINARY TUBERCULOSIS

I. Renal:	
Kidney and ureter	9
Bladder	4
II. Genital:	
A. Male—	
Prostate	1
Epididymis	3
Testis	1
B. Female—	
Uterus	
Ovaries and tubes	
III. Tuberculous bacilluria	5

The conclusion of 7% was derived from the ratio of 15 positive cases to the 214 studied. During this interval of time, 1230 individuals were treated at the sanatorium. Thus, considering the total population, the percentage of genito-urinary tuberculosis was 1.2%.

All organisms isolated were identified bacteriologically by their cultural characteristics and by guinea pig inoculations. From 1 woman a positive culture was subsequently identified as a smegma organism. The occurrence of non-tuberculous acid fast organisms in our survey was 1:29.

Clinical Course. Clinically, our cases can be divided into 3 separate groups:

1. *Clinical Genito-urinary Tuberculosis.* These were the cases which offered frank clinical signs: pyuria, hematuria, dysuria, enlarged testicle, or epididymis, etc. Tubercle bacilli were abundant and already in the plain smear of the 24-hour specimen. The patients had complaints, the clinical and laboratory findings were many.

Case Histories. V. 29, white male. In 1939 pleurisy with effusion which was tapped. In 1940 gradual onset of pain and burning on urination, frequency. Urine showed albumin. Urine cultures positive for tubercle bacilli; later smears of concentrates positive on direct examination. Cystoscopy: granulation tissue and ulceration at both ureteral openings, more so at right. Pyelography: left kidney disease. Nephrectomy performed. Specimen showed caseous-ulcerative tuberculosis of kidney and ureter.

M. 34, Philippine male. Had been admitted to another institution because of urinary distress but "work-up did not exclude the possibility of tuberculosis." Admitted to Otisville for chest condition and complaints of dysuria, frequency, and hematuria. Urine: alb. 4+, RBC, numerous WBC/HPF. Cystoscopy: ulceration of bladder and scarification of left ureter. Subsequent course: development of urinary retention, onset of abdominal tenderness. Laparotomy performed: perforation of posterior wall of bladder, tuberculous peritonitis. Transferred to the City. Left nephrectomy done. Patient recovered from the acute bladder disease.

2. *Subclinical Genito-urinary Tuberculosis.* On the other hand, there were the cases in which symptoms and complaints were vague or absent. Because of some abnormal content of the urine, 24-hour

specimens were cultured and tubercle bacilli found. Successive positive findings set the indication for cystoscopy and pyelography which revealed conclusive pathology.

These are the cases which were discovered early, and they differ from clinical genito-urinary tuberculosis only in degree and extent. All tuberculosis begins as minimal disease, and timeliness of diagnosis is desirable also in genito-urinary tuberculosis.

TABLE 3.—REPORTS ON GENITO-URINARY TUBERCULOSIS

Author		Type of material	Incidence of genito-urinary pathology	%
Harris	1930	Tuberculous bone lesions	16 of 43 adults	37
			9 of 67 children	13
Bumpus	1930	Urine from supposedly normal contralateral kidney	43 of 175	25
Rieder	1931	Mod. and far adv. cases	11 of 136	8
Menton	1932	Mod. and far adv. cases	1 of 76	1.3
Band	1935	Patients with extrapulmonary tuberculosis	25 of 174	14
Munro	1935	Mod. and far adv. cases	22 of 160	14
Saenz, etc.	1935	Mod. and far adv. cases	0 of 100	
Mack	1937	Patients with extrapulmonary tuberculosis	15 of 20	75
Miller, etc.	1939	General tuberculosis population	84 of 1316	6.6
Rosencrantz	1940	Mod. and far adv. cases	14 of 200	7
Morse, Scott	1940	General tuberculosis population	76 of 2049	3.8
Auerbach	1940	Autopsy material	127 of 1143	11

* Correspondence with the authors corrected this percentage from 28.

Case Histories. T. 25, white male. Admitted for chest condition. Urine: alb. 2+. Positive cultures. Intravenous pyelography revealed disease of left kidney. Nephrectomy performed and specimen showed caseous-ulcerative disease of left kidney.

M. 49, Japanese male. Past history of pleurisy with effusion, and gonorrhea. Urine on admission contained 2+ alb., clumps of WBC, and RBC. Urine cultures for tuberculosis set up. In view of history, an intermittent discharge was investigated for gonorrhea. Cultures positive on two occasions, complement fixation for gonorrhea: 5.9. Patient given several courses of sulfathiazole. Of 7 cultures for tubercle bacilli, 4 became positive. Pyelogram showed calcification in *right* kidney. Split specimens revealed tubercle bacilli coming from *left* kidney (positive culture and positive guinea pig inoculation). Diagnosis: calcified foci in right kidney, active focus in left; tuberculosis prostatitis activating old gonococcal infection. Transferred to city, where tuberculous epididymitis uncovered. Epididymectomy performed.

3. *Tuberculous Bacilluria, Occult Genito-urinary Tuberculosis.* Here again the indication for urologic examination was set by the laboratory. The excretion of tubercle bacilli was not constant, and clinical investigation could determine no causative pathology. Apparently, function was normal, and evidence of morbid change could not be revealed by the urologist. Yet, we must assume that a focus, or several foci, and these even bilaterally, had been established during Phase II of the patient's tuberculous history. When the foci heal, and they will heal as completely as do similar foci in the lungs,[1,3,6,7] the bacilluria is transitory. On the other hand, the foci may go on to ulceration and the case then becomes one of subclinical genito-urinary tuberculosis. These cases require constant check in order that surgical intervention be instituted before the appearance of frank clinical signs.

Case History. Five of our 15 cases were of this type. Anatomic pathology was not found, yet because of the repeated isolation of tubercle bacilli the literature has taught us to interpret these as authentic instances of renal tuberculosis. They are, then, the minimal cases heretofore overlooked unless "they had good luck to have their renal tuberculosis discovered in the preclinical or occult stage because they happened to be selected for inclusion in a research or enjoy the services of an exceptionally thorough clinical pathologist" (Dukes[4]). Whereas, the common location of an occult focus is in the kidney, the epididymis, etc., should not be overlooked.

Whereas *clinical* and *subclinical* excretory tuberculosis differ only in the extent of the symptomatology, we endeavor to stress the importance of the *tuberculous bacilluria* by grouping it with SUBCLINICAL form into PRECLINICAL genito-urinary tuberculosis.

Discussion. Pulmonary tuberculosis is very frequently complicated by urogenital tuberculosis (Table 3), in fact, more so than is generally accredited. Unfortunately, the diagnosis of urogenital tuberculosis is very often missed in the first stage of the disease because of its asymptomatic course.

The Pathogenesis of Urogenital Tuberculosis:

1. *Ascending*—mechanical factors or from the prostate.
2. *Direct Extension*—from the neighboring organs.
3. *Lymphatic.*
4. *Hematogenous*—the most frequent.

The most usual method of renal infection is by the hematogenous route. Renal tuberculosis is never a primary lesion but a complication of a tuberculous process localized elsewhere, the renal infection occurring when the primary focus in the body has already manifested itself. Renal tuberculosis as a hematogenous complication of this primary focus arises when the tubercle bacilli localize in the renal cortex and cause a simultaneous infection in both kidneys.[1,3,5,6] These foci may heal just as primary foci in the lung heal. When these foci break down, active renal tuberculosis occurs, the tubercle bacilli enter the urinary tubules and appear in the urine. "As the infection spreads, caseous foci are formed in the medullary pyramids and give rise to ulcerations of the overlying epithelial lining of the calyces in the renal pelvis, as a rule involving only one kidney."[5]

Secondly, in the evolution of an individual's disease, tuberculosis of any organ can break down and disseminate tubercle bacilli *via* the blood stream and set up a renal infection even after the individual had reached the tertiary period of Ranke (Fig. 1). Thus, excretory tuberculosis should be expected as a frequent occurrence.

A minority of authors contend that the tubercle bacilli may pass through a non-diseased kidney. However, generally authors believe that the finding of tubercle bacilli in the urine is indicative of renal tuberculosis provided that the genital system is ruled out. This conclusion has been borne out by a number of experiments. Medlar and Sasano[7] inoculated guinea pigs with tubercle bacilli, examined the urine, sacrificed the guinea pigs and demonstrated that in all cases showing tubercle bacilli in the urine, kidney pathology was always found. Munro and Bond studied 5 cases of bacil-

luria with no symptoms during life. In all 5 cases tuberculous foci were found in both kidneys. Spitzer and Williams[9] inoculated 103 guinea pigs with normal urine from patients with pulmonary tuberculosis. All pigs remained normal. However, in another series of 106 cases, 4 of the urinary specimens contained albumin, casts, or white blood cells. Of these, 4 of the guinea pigs died of tuberculosis.

"A true tubercle bacilluria does not exist, as an impartial review of the evidence in favor of and against such an occurrence reveals. The presence of tubercle bacilli in the urine signifies a specific lesion; and the ability to demonstrate it by clinical and laboratory methods is possible in practically all cases if they can be kept under observation sufficiently long."[5]

Morse and Scott[8] for the past 9 years routinely examined the urine of every patient in the Manitoba Sanatorium (2409). A culture of the urine was made in any case showing pus, albumin, or red blood cells. If the culture was doubtful, a guinea pig inoculation was carried out. These authors found that in 76 cases (3.8%), tubercle bacilli were present in the urine. Forty of the 76 cases had pulmonary tuberculosis, 12 had osseous lesions. In 5 cases, no extra-renal tuberculosis could be found. Nineteen patients had a bilateral involvement.

Our experience is reported to stress the frequency with which excretory tuberculosis can be uncovered in patients with comparatively little chest disease and without renal symptomatology. The tuberculous bacilluria is as important as the more advanced stages, for thus, an adequate régime can be instituted to treat the renal infection and progression to an unfavorable degree possibly avoided.

Beach and Schultz[2] followed 8 patients over different periods of time because varying numbers of pus cells and erythrocytes were found in bladder urine specimens. This follow-up over 8 to 15 years revealed that objective or subjective symptoms have not returned and in their absence the authors conclude that the tuberculous renal pathology from which these persons suffered at first has healed. The cases are reported in support of the contention that clinical healing of early renal tuberculosis does occur, especially if cessation or extensive anatomic changes have not occurred.

Conclusions. 1. Urinary findings of an increased number of white blood cells, albumin, or blood in a tuberculous individual should cause investigation for genito-urinary tuberculosis.

2. Concentrates of 24-hour urine specimens for tubercle bacilli supplemented by culture methods should be routine in every tuberculosis institution.

3. In a sanatorium population with comparatively little chest disease, of 214 cases investigated for excretory tuberculosis, 15 (7%) gave positive findings. Of these, 5 were in the form of a tuberculous bacilluria.

4. Genito-urinary tuberculosis can be divided into 3 clinical forms: (*a*) *Clinical*—symptoms are frank and many, pathologic change is obvious; (*b*) *Subclinical*—in which laboratory findings set the indications for urologic work-up; pathologic condition is then uncovered; (*c*) *Tubercu-*

lous bacilluria—the form in which urine cultures for tubercle bacilli are positive, yet pathologic change though present, is not manifest.

5. *The tuberculous bacilluria* and the *subclinical form* have been grouped together into *preclinical genito-urinary tuberculosis.*

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STUDIES ON 2-SULFANILAMIDO-4-METHYL-PYRIMIDINE (SULFAMERAZINE, SULFAMETHYLDIAZINE) IN MAN

III. The Treatment of Meningococcic Meningitis.*

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Sulfamerazine (2-sulfanilamido-4-methyl-pyrimidine), synthesized by several groups of investigators,[8,10] is one of several methyl homologues of sulfadiazine. The pharmacologic and toxicologic behavior of this compound in laboratory animals,[11] and its therapeutic efficacy against a variety of experimental infections[8] suggested that this drug had definite clinical potentialities. Studies in man have shown that higher blood levels are attained more rapidly and are sustained for a longer period of time following the oral administration of sulfamerazine than after the ingestion of similar amounts of sulfadiazine.[5,6] Results of preliminary studies suggest that the toxicity of sulfamerazine to man is comparable to that of the sulfonamides in common use at the present time.[2]

These studies of the behavior of sulfamerazine suggested that its use in the treatment of meningococcic meningitis might prove advantageous. This disease was epidemic in Philadelphia in the past winter

* From the Philadelphia General Hospital Committee for the Study of Pneumonia, which also includes J. H. Clark, M.D., and J. G. Reinhold, Ph.D. Miss M. E. Murphy and Miss J. O'Laughlin rendered valuable technical assistance.

and ample opportunity was provided for the clinical trial of sulfamerazine. Up to the time of writing, 45 patients, suffering from meningococcic meningitis,* have been treated with sulfamerazine.† The relevant data are shown in Table 1. Thirty-six of the patients were adults, ranging in age from 13 to 77 years, and the remaining 9 were children of 1 to 11 years. All were gravely ill and presented typical clinical manifestations of meningitis. The cerebrospinal fluid was turbid in every instance but one (Case 13), in which the lumbar puncture was performed 8 hours after onset of symptoms. Bacteriologic studies were made throughout the investigation. Spinal fluid cultures were positive for the meningococcus in all but 7 cases and in these the diagnosis was supported by the finding of typical organisms on direct smear. No explanation for our failure to obtain growth in these 7 cases is available. In most instances, a luxuriant growth was obtained within 16 hours. On the basis of our experience this favorable result was due in a large measure to the routine use of capneic incubation.[8] Blood cultures were made routinely and meningococci were found in 8 cases (17.8%).

In general, the following dosage of sulfamerazine was employed. The initial dose was always given intravenously as sulfamerazine sodium (5% solution in sterile distilled water), adults receiving 3 gm. and children 1 to 2 gm. This dose was immediately followed by sulfamerazine orally, adults receiving 1 gm. every 4 hours, children receiving 0.25 gm. to 1 gm. every 6 hours. In delirious or comatose cases the drug was administered by nasal tube until the patient was capable of taking medication by mouth. Sulfamerazine dosage was continued in full amounts, unless toxic manifestations developed, until the patient appeared entirely well clinically. In the successfully treated group the average total dosage of the drug for the adults was 56.4 gm., given over an average period of 9.5 days, ranging from 5 to 16 days; the children received an average total dosage of 19.3 gm., over an average period of 8.6 days, ranging from 6 to 14 days. In 5 of the patients (Cases 8, 24, 30, 41, and 44) intravenous antimeningococcic serum was employed in addition to sulfamerazine, with one death (Case 30). The total amount of serum used in each case was generally small, varying from 30 to 50 cc. Whether the successfully treated cases in this combined therapy group would have recovered without the additional use of serum cannot be stated definitely. It is our impression, however, that the drug alone would have achieved the same results. Fluids were given liberally, usually 2000 to 3000 cc. a day. None of the patients received alkali therapy.

Determinations of the amount of free drug in the blood were made at frequent intervals in most of the patients. For those receiving 6 gm. of sulfamerazine per day, plasma concentrations of free drug were as follows in 32 cases: First 12 to 24 hours, 13.4 mg. per 100 cc.; 2d day,

* These patients were treated on the Medical and Pediatric Wards of the Philadelphia General Hospital (37 cases) and the Hospitals of the University of Pennsylvania (8 cases).

† Sharp & Dohme Laboratories, Glenolden, Pa., kindly supplied the sulfamerazine used in this study.

TABLE 1.—ANALYSIS OF DATA ON 45 CASES OF MENINGOCOCCIC MENINGITIS

No.	Age. Yr.	Sex	Duration (days)	Clinical condition on admission	Initial cerebrospinal fluid			Blood culture	Sulfamerizine therapy			Course of treatment (days)		Results and comments
					Turbidity*	Smear	Culture		Dur. (days)	Total dose gm.	Toxicity	Clinical improv.	Afebrile	
1	36	M	4	Unconscious	+	+	+	0	13	81	....	1	6	Rec.
2	33	F	2	Stuporous	++++	+	0	0	8	42	....	4	4	Rec.; transient arthritis, onset 8th day
3	41	M	?	Moribund	++	+	+	0	3	20	....	..	..	Died on 3d day
4	51	M	2	Unconscious	++++	+	+	0	11	69	....	2	4	Rec.; transient Bell's palsy, onset 9th day
5	47	M	3	Unconscious	++++	+	+	0	11	63	....	2	6	Rec.
6	22	M	3	Delirious	++	+	+	0	11	63	....	2	6	Rec.
7	46	M	3	Delirious	++++	+	+	0	11	63	....	2	4	Rec.
8	37	M	4	Unconscious	++	+	+	0	14	83	Fever 13th day	4	12	Rec.; barbiturate poisoning, acute alcoholism, 45 cc. antimeningococcic serum, i.v.
9	22	M	2	Drowsy	++++	+	+	+	10	63	....	2	4	Rec.
10	16	F	4	Semiconscious	+++	+	+	0	10	46	Rash 10th day	3	3	Rec.
11	23	M	5	Delirious	++	+	+	0	12	77	....	4	5	Rec.
12	14	M	3	Drowsy	++	+	+	0	8	51	Rash 9th day	1	1	Rec.; transient ocular palsy on admission
13	28	F	1	Drowsy	Clear	−	+	+	5	29	Rash 5th day	1	1	Rec.
14	16	M	5	Stuporous	++++	+	+	0	9	47	Fever 8th day	3	4	Rec.
15	42	M	3	Semiconscious	++++	+	+	0	10	81	....	4	5	Rec.
16	23	M	2	Delirious	++++	+	+	+	9	57	....	1	1	Rec.
17	23	F	3	Unconscious	+++	+	+	0	11	69	....	2	12	Rec.; transient polyarthritis onset 3d day
18	48	M	3	Drowsy	+++	+	0	0	7	45	....	1	6	Rec.
19	18	F	2	Unconscious	++++	+	+	+	14	77	Acute loin pain and hematuria 14th day	4	14	Rec.; transient arthritis, onset 8th day
20	50	F	4	Stuporous	++++	+	+	+	16	51	....	4	5	Rec.
21	17	M	2	Delirious	+++	+	+	0	10	39	....	2	9	Rec.
22	32	M	6	Drowsy	++	+	+	0	10	38	....	2	3	Rec.
23	9	M	1	Stuporous	+++	+	+	+	10	21	....	1	1	Rec.
24	5	M	2	Unconscious	+++	+	+	+	6	15	....	2	3	Rec.; 45 cc. antimeningococcic serum, i.v.
25	3	F	4	Stuporous	++	+	+	0	14	13	....	2	3	Rec.
26	24	M	5	Drowsy	++++	+	+	0	7	45	....	1	3	Rec.
27	11	M	3	Drowsy	+++	+	0	0	7	31	....	1	3	Rec.; transient ocular palsy on admission
28	30	F	5	Delirious	++	−	+	0	11	69	....	5	14	Rec.; transient polyarthritis on admission
29	29	M	3	Drowsy	++++	+	0	0	5	33	....	1	1	Rec.
30	36	M	3	Unconscious	+++	+	+	0	13	105	....	..	..	Died on 21st day, 45 cc. antimeningococcic serum, i.v.
31	7	F	3	Semiconscious	++++	+	+	+	8	17	....	4	11	Rec.
32	1	M	3	Drowsy	++++	+	+	0	8	9	....	2	4	Rec.
33	77	M	?	Unconscious	+	+	+	0	1	9	....	..	..	Died 23 hrs. after onset of treatment
34	28	M	1	Stuporous	+++	+	+	0	7	45	Fever 7th day	1	6	Rec.
35	43	M	3	Drowsy	+++	+	0	0	4	27	....	1	1	Rec.
36	9	M	2	Drowsy	++++	+	0	0	9	25	Rash 9th day	1	2	Rec.
37	25	M	1	Semiconscious	+++	+	+	0	14	83	Rash 14th day	3	6	Rec.
38	2	M	2	Stuporous	++	+	+	0	8	21	....	2	2	Rec.; transient ocular palsy on admission
39	48	F	6	Unconscious	++	+	+	0	9	51	Rash 9th day	2	6	Rec.
40	41	F	2	Stuporous	+	+	0	0	9	56	Fever 8th day	2	3	Rec.
41	16	M	2	Drowsy	++	+	+	0	11	69	....	3	10	Rec.: 50 cc. antimeningococcic serum, i.v.
42	26	F	2	Drowsy	++	+	+	0	6	39	....	1	9	Rec.
43	13	F	2	Semiconscious	+++	+	+	0	9	54	....	1	3	Rec.
44	34	F	4	Semiconscious	+++	+	+	0	9	57	....	3	6	Rec.; 30 cc. antimeningococcic serum, i.v.
45	3	M	4	Stuporous	+++	+	+	0	7	22	....	2	7	Rec.

* Turbidity: + = less than 1000 cells; ++ = 1000 to 5000 cells; +++ = 5000 to 10,000 cells; ++++ = more than 10,000 cells.

Rec., recovered.

15.7 mg. per 100 cc.; 3d day, 16.5 mg. per 100 cc.; 4th day, 16.0 mg. per 100 cc.; 5th day, 15.5 mg. per 100 cc. Result of blood analyses will be reported in detail later. Lumbar punctures were done on admission for diagnostic purposes, but the procedure was not repeated unless signs of increased intracranial pressure were present or the patient failed to respond to treatment, in which case further study of the cerebrospinal fluid seemed advisable. During the course of this study, however, we did obtain cerebrospinal fluid from 28 patients in order to determine the amount of free drug present. It was found that the mean concentration of the drug in the cerebrospinal fluid was 42.1% of the mean concentration in the plasma.

Results. In this group of 45 patients with meningococcic meningitis treated with sulfamerazine there were 3 deaths (6.7% mortality). Of the fatal cases, one (Case 3) was a 41-year old white man who was moribund on admission and died 3 days later without showing any response to treatment. No history as to the duration of illness was obtainable in this case. The second death (Case 30) was a colored male, aged 36 years, admitted on the 3d day of his disease. At the time of admission the patient was unconscious and had a right-sided torticollis, presumably of cerebral origin. Initial examination of the cerebrospinal fluid revealed marked turbidity, many meningococci on smear, and a positive culture. Subsequent cultures of the fluid on the 5th and 9th days of treatment were sterile. This patient received a total of 105 gm. of sulfamerazine over a period of 13 days and 45 cc. of antimeningococcic serum intravenously on the 3d day. Although the negative cerebrospinal fluid findings were suggestive of some response to the drug, the clinical course was progressively downhill and the patient died on the 21st day. The pertinent autopsy findings were referable to the brain.* There was no gross evidence of meningitis. Areas of recent softening were observed, superimposed on calcification of vessels in the globus pallidum. This lesion was considered to be due to chronic anoxia, to which the meningitis might have been a contributing factor. This could also have accounted for the clinical torticollis and persistent unconsciousness. The third death (Case 33) was a 77 year old white man who was unconscious on admission and died 23 hours after the onset of therapy, having received only 9 gm. of sulfamerazine.

For the successfully treated group, definite clinical improvement, manifested mainly by mental clarity, was apparent within 48 hours of drug therapy in 29 (70%) of the cases, although fever usually persisted for several days following the initial response. The average time observed before the recovered group became afebrile was 5.2 days, ranging from 1 to 14 days. Complications of the disease were observed in 4 patients on admission, 3 with ocular palsies (Cases 12, 27, and 38) and 1 with polyarthritis (Case 28). During the course of treatment, 1 patient (Case 4) developed Bell's palsy, and 3 patients developed

* Examination of the brain was performed by Helena E. Riggs, M.D., Division of Neuropathology, Laboratories, Philadelphia General Hospital.

acute arthritis (Cases 3, 17, and 19). All complications were transient and recovery was complete.

Toxicity. Toxic reactions, attributable to the drug, were noted in 11 patients. Six (Cases 10, 12, 13, 36, 37 and 39) developed skin rashes on the 10th, 9th, 5th, 9th, 14th and 9th days of treatment following 46, 51, 29, 25, 83 and 51 gm. of sulfamerazine respectively. Drug fever was observed in 4 patients (Cases 8, 14, 34 and 40) on the 13th, 8th, 7th and 8th days of treatment after the administration of 77, 41, 45 and 50 gm. of the drug respectively. One patient (Case 19) experienced acute loin pain and gross hematuria on the 14th day of treatment, after having received 77 gm. of sulfamerazine. This case has been reported elsewhere in detail.[2] All of the patients in this sub-group recovered despite the fact that sulfamerazine was discontinued in each instance with the diagnosis of drug toxicity.

Comments. The mortality of 6.7% in this group of 45 cases of meningococcic meningitis treated with sulfamerazine is to be compared with that of 57.5% occurring in 40 cases of this disease observed at the Philadelphia General Hospital during 1935, 1936 and 1937,[7] and with that of 40% in 50 patients reported in 1942.[1] Also the results in this study compare favorably with those recently reported in which sulfadiazine was employed (12.5% mortality).[3] Due to unavoidable circumstances it was not practical to treat any of the cases in this series other than with sulfamerazine.

Toxic reactions due to the drug were more frequent in these patients receiving sulfamerazine than in those previously treated by means of sulfadiazine.[3] However, in a larger group of pneumonia cases in whom sulfamerazine was used over shorter periods of time and in smaller daily doses, the incidence of drug toxicity was negligible.[4]

During the course of this investigation there were 6 additional patients treated with sulfamerazine in whom the diagnosis of meningococcic meningitis was based in each instance on a typical clinical picture, increased spinal fluid pressure, and a turbid cerebrospinal fluid with a high polymorphonuclear cell count. Since the clinical diagnosis in these cases was not substantiated by convincing bacteriologic evidence, we have excluded them from this report, although we believe they represent true cases of the disease. These 6 cases recovered. In addition to the above, there were 3 patients with proven meningococcus meningitis who were moribund on admission and died before sulfamerazine therapy could be instituted.

Summary. 1. Sulfamerazine therapy has been employed in 45 consecutive cases of meningococcic meningitis. There were 3 deaths in the series (6.7% mortality).

2. Definite clinical improvement with return of mental clarity occurred in 70% of the patients within 48 hours. The average time observed for the return to normal temperature was 5.2 days.

3. Toxic reactions attributable to sulfamerazine, occurring in each instance after the 5th day of treatment, were noted in 11 patients.

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STUDIES ON 2-SULFANILAMIDO-4-METHYL-PYRIMIDINE (SULFAMERAZINE, SULFAMETHYLDIAZINE) IN MAN

IV. THE TREATMENT OF PNEUMOCOCCIC PNEUMONIA*

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IN the preceding article we reviewed the therapeutic effectiveness of sulfamerazine (2-sulfanilamido-4-methyl-pyrimidine, sulfamethyldiazine) against experimental infection, discussed the behavior of the drug in man, and presented observations on its use in the treatment of meningococcic meningitis. The present paper deals primarily with the treatment of pneumococcic pneumonia with sulfamerazine.†

Organization of the Study. A prime requirement in the evaluation of a new chemotherapeutic agent for the treatment of pneumonia is to compare it with a proven form of therapy. Therefore, from the beginning of this study (January 1, 1943), as in previous studies of sulfonamide therapy in pneumonia, the medical services‡ in the hospital were divided into two therapeutic groups, one receiving sulfamerazine, and a control group, consisting of an equal number

* From the Philadelphia General Hospital Committee for the Study of Pneumonia, which also includes J. G. Reinhold, PH.D., and S. B. Rose, M.D.

† Sharp & Dohme Laboratories, Glenolden, Pa., kindly supplied the sulfamerazine used in this study.

‡ Clinical facilities were given us for this study by the following Chiefs of Service: Drs. Frieda Baumann, R. S. Boles, C. L. Brown, Thomas Klein, D. W. Kramer, D. N. Kremer, W. G. Leaman, Jr., S. A. Loewenberg, and T. G. Schnabel.

of patients with pneumonia, receiving sulfadiazine. For this report we have taken the first 80 cases in each therapeutic group. In general, the distribution of age and sex (Table 1), and the day of disease on which treatment was begun (Table 2) were comparable in the two groups.

TABLE 1.—MORTALITY ACCORDING TO AGE AND SEX GROUPS

Age group (yrs.)	Sulfamerazine treated		Sulfadiazine treated	
	No.	Died	No.	Died
12–19	10	..	8	
20–29	11	..	14	
30–39	23	1	15	1
40–49	16	2	21	2
50–59	10	2	14	4
60 and over	10	1	8	1
Sex				
Male	70	5	73	8
Females	10	1	7	

TABLE 2.—MORTALITY IN RELATION TO DAY OF DISEASE ON WHICH TREATMENT WAS BEGUN

Day of disease treatment started	Sulfamerazine treated			Sulfadiazine treated		
	No.		Died	No.		Died
1	8	67.5	1	6	55.0	
2	16		1	15		
3	15		..	10		
4	15		..	13		2
5	11	32.5	1	10	45.0	1
6	7		..	9		
7	5		2	10		1
7+	3		1	7		4

The diagnosis of typical pneumonia was established in every case by the natural history of the disease and the findings on physical examination. When indicated, the diagnosis was confirmed by roentgen studies. Pneumococci were isolated from the sputum or blood stream of 134 patients (70 sulfamerazine, 64 sulfadiazine). A specific type of pneumococcus was recovered from 73 of the cases (35 sulfamerazine, 41 sulfadiazine). A higher incidence of typed pneumococci would undoubtedly have been obtained had we employed typing as a routine procedure. Instead, typing was done on those pneumococci isolated from the blood stream and those found in the sputum samples of seriously ill patients. In 26 cases (10 sulfamerazine, 16 sulfadiazine) we were unable to confirm the clinical diagnosis of a pneumococcic infection by convincing bacteriologic studies because sputum samples were either unobtainable or else failed to yield pneumococci on stained smear and culture of the sputum. Repeated blood counts, urinalyses, blood cultures, and determinations of free and total drug in the blood plasma were made in all patients receiving sulfamerazine or sulfadiazine. Other laboratory studies were carried out as indicated.

Dosage. With sulfamerazine, two dosage schedules were employed: an initial 3 gm. dose by mouth was followed by 1 gm. every 6 hours in the first 23 patients treated; the remaining 57 patients received the 1 gm. dose every 8 hours. With sulfadiazine, the dose schedule was the same as that used with sulfamerazine, except that all of the patients in this group were given 1 gm. every 6 hours. The average total dosage for the successfully treated patients receiving sulfamerazine was 24.4 gm. (4 gm. daily for 5.6 days), or 22.7 gm. (3 gm. daily for 6.5 days), as compared with 30.8 gm. for the sulfadiazine treated group (4 gm. daily for 7 days). In certain instances, when a rapid elevation of the blood level of either drug was desired, the initial 3 gm. of drug was given by vein. A 5% solution of the sodium salt of sulfamerazine or sulfadiazine in sterile distilled water was employed in such cases. When sulfa-

merazine or sulfadiazine failed to bring about a favorable clinical response within 36 to 48 hours, the patient was usually given specific serum. Of the 160 patients included in this report, 12 (6 sulfamerazine, 6 sulfadiazine) received serum together with chemotherapy. In addition, a single case in each therapeutic group was given penicillin. All patients received at least 2500 cc. of fluid during each 24-hour period, and those treated with sulfadiazine were given either sodium bicarbonate or sodium citrate[8] in an amount equal to that of the drug.

Therapeutic Results. The results of treatment in the 2 therapeutic groups are shown in Table 3. Of the patients treated with sulfamerazine, 6 died, and in the sulfadiazine treated group, 8 died, the mortality rates being 7.5% and 10%, respectively. Eighteen patients with bacteremia were treated with sulfamerazine and 6 of these died (33.3% mortality). In the sulfadiazine treated group, 4 of the 22 bacteremic patients died (18.2% mortality). The fatal cases are analyzed in Table 4.

TABLE 3.—DISTRIBUTION OF TYPES, BACTEREMIA, AND MORTALITY RATES

	Sulfamerazine treated				Sulfadiazine treated			
	All cases		Bacteremic cases		All cases		Bacteremic cases	
Type	No.	Died	No.	Died	No.	Died	No.	Died
I	6	..	3	..	10	2	4	1
II	3	1	1	1	6	..	4	
III	3	1	2	1	2	..	1	
Group IV	21	4	12	4	24	3	13	3
Others	47	..	..	..	38	3		
Total	80	6	18	6	80	8	22	4
Mortality %	7.5		33.3		10.0		18.2	

In evaluating any therapeutic agent clinically, one must consider the effect of the agent on the course of the disease as well as its influence on the mortality incidence. As shown in Table 5, a critical drop in temperature occurred within 24 hours in 51.4% of the patients treated with sulfamerazine, as compared with 30.6% of the patients in the sulfadiazine treated group. Within 48 hours, 77.1% of the patients receiving sulfamerazine and 66.7% of those treated with sulfadiazine showed a critical drop in temperature. The temperature returned to normal within 24 hours in 17.5% of the patients in the sulfamerazine treated group and in 15.3% of the patients receiving sulfadiazine. Within 72 hours a return to normal temperature occurred in 72.9% and 59.8% of patients in the sulfamerazine and sulfadiazine groups respectively.

Complications. The incidence of complications was low (7 cases, 4.4%) for the entire series of 160 patients (Table 6), 2 (2.5%) occurring in the sulfamerazine and 5 (6.3%) in the sulfadiazine treated group. Three cases (1 sulfamerazine, 2 sulfadiazine) developed endocarditis; all of these patients died. Empyema occurred in 3 sulfadiazine treated cases with no deaths. A single patient receiving sulfamerazine developed meningitis and failed to recover. In this sub-group all patients with the exception of the case with meningitis were started on drug therapy after the 6th day of the disease.

TABLE 4.—ANALYSIS OF FATAL CASES

No.	Age (yrs.)	Day of disease treatment begun	Type	Blood culture	No. of lobes involved	Total drug (gm.)	Total serum (units)	Remarks
				Sulfamerazine				
1	46	1	XXV	Pos.	3	56	200,000	No response to treatment; aut.: lobar pneumonia of entire right lung
2	61	5	XVI	Pos.	2	22		Cardiac failure on adm.; no autopsy
3	41	2	II	Pos.	2	33	100,000	Rt. phthisis bulbæ, pansinusitis on adm.; purulent ophthalmitis 4th day; convulsions on 13th day; aut.: resolving lobar pneumonia, meningitis, purulent sphenoiditis
4	33	7	VIII	Pos.	5	15	100,000	Chr. alcoholism; no response to treatment; aut.: lobar pneumonia of all 5 lobes
5	51	28	XII	Pos.	3	36	100,000	Clin. picture of ac. bact. endocarditis; serum sensitive; received penicillin 250,000 units; no aut.
6	58	7	III	Pos.	3	8	100,000	Moribund on adm. with leukopenia; aut.: lobar pneumonia of both lower lobes.
				Sulfadiazine				
1	50	10	VII	Pos.	2	19		Responded well to treatment; developed ac. pulm. edema, sudden death; no aut.
2	51	4		Neg.	1	8		Moribund on adm.; no response to treatment; died in 36 hrs.; aut.: lobar pneum.
3	44	7	I	Neg.	4	7	100,000	Moribund on adm.; no response to treatment; died in 30 hrs.; aut.: lobar pneum. of entire right lung and left lower lobe
4	68	20		Neg.	2	27		Responded well to treatment; drug stopped on 6th day; died on 9th day, cardiac decomp.; no autopsy
5	54	5	XI	Pos.	4	11		Moribund on adm.; no response to treatment; aut.: lobar pneumonia involving 4 lobes
6	53	21	XXIV	Pos.	3	23	120,000	No response to treatment; penicillin 160,000 units; aut.: resolving lobar pneum., ac. bact. endocarditis
7	34	4			1	22		Ac. pulm. edema on adm.; on response to treatment; aut.: lobar pneumonia
8	45	10	I	Pos.	3	51		Diabetes mellitus; initial response to treatment, then relapse; aut.: resolving lobar pneumonia, ac. bact. endocarditis

TABLE 5.—EFFECT OF TREATMENT ON TEMPERATURE RESPONSE (146 CASES)*

	Sulfamerazine treated			Sulfadiazine treated		
	No.	%		No.	%	
Critical fall in temperature:						
Within 24 hours	38	51.4	77.1	22	30.6	66.7
Within 48 hours	19	25.7		26	36.1	
Over 48 hours	17	22.9		24	33.3	
Temperature at normal level:						
Within 24 hours	13	17.5	72.9	11	15.3	59.8
Within 48 hours	28	37.9		20	27.8	
Within 72 hours	13	17.5		12	16.7	
Over 72 hours	20	27.1		29	40.2	

* All deaths excluded.

TABLE 6.—INCIDENCE OF COMPLICATIONS AND TOXIC REACTIONS (160 CASES)

	Sulfamerazine treated		Sulfadiazine treated	
Complications	No.	%	No.	%
Endocarditis	1	1.3	2	2.5
Empyema	..	..	3	3.8
Meningitis	1	1.3		
Total	2	2.5	5	6.3
Toxic reactions				
Vomiting	2	2.5	2	2.5
Hematuria (micro.)	3	3.8	4	5.0
Psychosis	1	1.3		
Drug fever	1	1.3	2	2.5
Total	7	8.9	8	10.0

Toxic Reactions. In this series of 160 patients the incidence of toxic reactions attributable to either drug was small (9.4%) and comparable for the two therapeutic groups (Table 6). Vomiting was noted in 2 patients in each group, but in no patient was it necessary to stop drug therapy since the symptom was transient. Microscopic hematuria was encountered in 3 patients receiving sulfamerazine and in 4 patients treated with sulfadiazine. Crystalluria was detected in approximately 15% of each group. No cases of gross hematuria, anuria, or renal pain were observed with either drug. The diagnosis of a drug psychosis was made in a single patient who was treated with sulfamerazine. Drug fever developed in 3 patients (1 sulfamerazine, 2 sulfadiazine). Repeated blood studies showed little evidence of a marked reduction in hemoglobin, red blood cell, or white blood cell counts that could be attributed to either drug.* No skin rashes were observed.

Blood Concentration of Sulfamerazine and Sulfadiazine.[7] The average concentration of free sulfamerazine in plasma as shown by daily determinations during the entire period of treatment was 12.7 mg. per 100 cc. (4 gm. daily), and 10.9 mg. per 100 cc. (3 gm. daily), compared with an average concentration of free sulfadiazine in plasma of 7.9 mg. per 100 cc. (4 gm. daily).

Comment. Sulfamerazine and sulfadiazine are both effective drugs in the treatment of pneumococcic pneumonia. The mortality of 7.5% in the sulfamerazine group and 10% in the sulfadiazine group compare favorably when all of the factors influencing fatality are considered.[3] Clinical response, as manifested by a fall in temperature, was slightly more in evidence with sulfamerazine than with sulfadiazine; and this, we believe, may be related to the more rapid rise and higher concentrations in blood attained by sulfamerazine as compared with sulfadiazine.[5,6]

In both therapeutic groups there was a low incidence of toxic manifestations (none of which was serious) following the use of these drugs. It is to be remembered, however, that the hazard of severe toxic reactions increases with the prolonged use of both sulfamerazine[1,4] and sulfadiazine.[2] In this series of pneumonia cases most patients required no more than 20 to 30 gm. of either drug, extending over a period of approximately 6 days. We believe that this explains in part the low incidence of toxicity encountered in this study.

Summary. 1. Sulfamerazine was given to 80 adult patients with pneumococcic pneumonia and the response was compared with that of a control series of 80 adult patients treated with sulfadiazine.

2. Mortality in the two therapeutic groups showed no significant difference (sulfamerazine 7.5%, sulfadiazine 10%).

3. Sulfamerazine tended to lower the temperature somewhat more rapidly than did sulfadiazine; however, the duration of chemotherapy and the incidence of complications were essentially the same for the two groups.

* Dr. Walter J. Crocker, Chief of the Division of Clinical Pathology of the Laboratories of the Philadelphia General Hospital, coöperated with the authors in this phase of the work.

4. The incidence of toxic reactions was low and comparable for both sulfamerazine and sulfadiazine. No serious reactions were encountered with either drug.

5. The group treated with sulfamerazine showed higher plasma concentration of free drug than did the group receiving larger or equivalent amounts of sulfadiazine.

Valuable assistance in the conduction of this study was rendered by Miss Rita Fenwick, R.N.

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TISSUE CULTURE STUDIES ON CYTOTOXICITY OF BACTERICIDAL AGENTS

III. Cytotoxic and Antibacterial Activity of Gramicidin and Penicillin; Comparison With Other Germicides

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In the first paper of this series[6] a report was made of the relative toxicity of certain bactericidal agents as determined by their influence on the extent of migration of lymphocytes in tissue culture preparations. We reported also on the antibacterial activity of these agents on Gram-positive cocci growing in a tissue culture medium similar to that used in the toxicity tests.[4,5] It was of interest to compare the bactericidal agents of microbial origin with other antiseptics by the use of these methods. Many germicides known to be useful for sterilization of the skin or other surfaces are not effective against bacteria in the presence of serum and tissue extract. Likewise, many germicides are quite toxic for tissue cells although they may be used safely on the skin. As a result, the choice of germicides that might be compared with gramicidin and penicillin under these conditions was limited. Merthiolate was included because it prevented the growth of bacteria under the conditions of the test, even though it is known that the tissue toxicity of the mercurial compounds as a class is rather high. One

member of the sulfonamide group of drugs was tested although it was assumed that the inhibitors present in the tissue culture medium would affect its activity greatly. The other two germicides studied were zephiran* and phemerol,* two synthetic detergents, both of them being cationic, quaternary ammonium compounds.

The results of cytotoxicity tests with penicillin were taken from the first paper of this series.[6] These tests were not repeated because they required the use of rather large amounts of penicillin. The same sample of penicillin was used in these tests and in the bactericidal tests reported in the present study.

Experimental. The method of determining the toxicity of each test substance has been described elsewhere.[5] Tissue was taken under sterile conditions from the mesenteric lymph node of the rabbit. Explants approximately 1.5 mm. across were carefully matched for size and shape and were divided into three similar groups each containing 12 fragments. One group of fragments was used in the control series of cultures and the second and third groups were used in the test series. One test was made with each of several different concentrations of each germicide. It was convenient to test only two dilutions of the same germicide or a single dilution of each of two germicides in the same experiment.

The penicillin used in these experiments had a potency of 35 Oxford units per mg. Solutions of penicillin in Tyrode's solution were prepared and stored in solid carbon dioxide. Stock solutions and suspensions of gramicidin were prepared as previously described. Dilutions of merthiolate, zephiran and phemerol were made from the commercial preparations containing 0.1% of each drug in aqueous solutions. Solutions containing sodium sulfathiazole were made in Tyrode's solution. Solutions containing germicides were brought to pH 7.6 just before the material was added to the culture medium.

Each culture was planted on a 22 mm. round coverslip and consisted of an explant of lymph node placed in the center of a clot consisting of 1 drop of heparinized rabbit's plasma and 3 drops of a rabbit's serum extract of chick embryos. An appropriate dilution of the germicide to be tested was added to the tissue extract at the time the culture was made in an amount equal to a tenth of the volume of the final tissue culture clot. A similar solution not containing the drug (Tyrode's solution, sterile distilled water or, in the case of gramicidin, Tyrode's solution containing ethyl alcohol) was added to cultures of the control series. Cultures were incubated as lying drops at 37° C. for 24 hours. At the end of the period of incubation the extent of migration of lymphocytes was determined by the use of an ocular micrometer at a magnification × 60. The distance from the edge of the original explant to the outer limit of migrating cells was measured at the widest part of the migration zone. The mean migration was determined for each group of 12 fragments and the values obtained for the test series were compared with those of the control series and were expressed in terms of percentage of inhibition.

The same kind of tissue culture medium was used in the bactericidal tests. Three strains of Gram-positive cocci were employed because they represent species of bacteria commonly found in local pyogenic infections. The strain of *D. pneumoniæ* Type VII was obtained from material removed from an empyema cavity and the strains of *S. aureus* and *S. pyogenes* were obtained from the blood of patients suffering from bacteremia. The strains of *D. pneumoniæ* Type VII and *S. pyogenes* were grown on Hartley's horse muscle digest broth containing 10% of horse serum. The strain of *S. aureus* was maintained on Hartley's broth alone. Dilutions of the bacterial cultures were made in

*Zephiran is the trade name for alkyl dimethyl benzyl ammonium chloride (alkyl = C_8 to C_{18}), Alba Pharmaceutical Company. Phemerol is para-tertiary-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride monohydrate, Parke, Davis & Company.

Hartley's broth and added to the tissue extract in the proportion of 1 part of a suspension of bacteria in broth to 40 parts of tissue extract. A sufficient amount of the original culture was used to cause the appearance of 20 or more bacterial colonies in each of the control cultures.

Dilutions of the germicide in Tyrode's solution were added to both the heparinized rabbit's plasma and tissue extract at the time the cultures were made. One part of solution containing germicide was added to 9 parts of plasma or tissue extract. Similar amounts of a corresponding solution not containing germicide were added to control cultures. Each culture consisted of 1 drop of plasma and 3 drops of tissue extract placed on a 22 mm. round coverslip. Four cultures were prepared for each experimental condition. Cultures were incubated at 37° C. for 48 hours. At this time the presence of bacterial colonies was determined by examination with a dissection microscope at ×7 magnification. The least amount of germicide that would completely prevent the appearance of bacterial colonies in all four cultures after incubation for 48 hours was determined for each bacterial strain tested. Two independent determinations of the end-point were made for each germicide.

Results. The results obtained in the tests on cytotoxicity are presented in Figure 1. Although considerable variation may exist in the determination of the percentage of inhibition taking place for any particular amount of germicide used, the general relationship between these drugs is fairly represented in the figure. Merthiolate is seen to be the most toxic. The use of 60 μg. per cc. of the drug produced a 72% inhibition of the migration of lymphocytes in 24 hours. Zephiran and phemerol were next in order of toxicity. The use of 100 μg. per cc. of gramicidin produced but a 24% inhibition. Penicillin and sodium sulfathiazole were the least toxic, it being necessary to use several hundred μg. per cc. of each before any effect was noted.

TABLE 1.—AMOUNT OF GERMICIDE (MICROGRAMS PER CC.) CAUSING INHIBITION OF BACTERIA

Germicide	Organism*		
	D. pneumoniæ Type VII	*S. aureus*	*S. pyogenes*
Penicillin	3.0	5	5
Gramicidin	0.6	100	10
Merthiolate	25.0	50	50
Zephiran	25.0	20	8
Phemerol	25.0	16	10
Sodium sulfathiazole	1000	Inhibition†	Inhibition†

* Two experiments were carried out for each germicide on each organism.
† Not complete with 2500.

The results of bactericidal tests are presented in Table 1. Penicillin was very effective against all three bacterial strains. Small amounts of gramicidin prevented the growth of pneumococci and hemolytic streptococci but comparatively large amounts were necessary to prevent the growth of staphylococci. Gramicidin has been found to be even less inhibitory for five other strains of *S. aureus* that we have tested. On the other hand, zephiran and phemerol were quite effective against *S. aureus* as well as against the other two test organisms. Merthiolate was as active as the two detergents in preventing the growth of pneumococci but larger amounts were needed to inhibit staphylococci and hemolytic streptococci. Sodium sulfathiazole was relatively ineffective against *S. aureus* and *S. pyogenes* and it was

necessary to use 0.1% of this drug to prevent the growth of pneumococci.

Under these conditions penicillin again was found to be superior to other germicides because of its bactericidal activity against Gram-positive cocci and its low toxicity for tissues. The cytotoxicity of the two detergents tested was not very much greater than that of gramicidin and their bactericidal activity compares favorably with that of gramicidin.

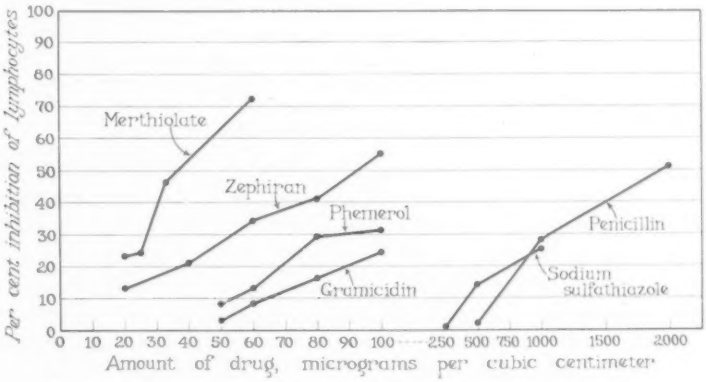

FIG. 1.—Cytotoxicity of various concentrations of bactericidal agents.

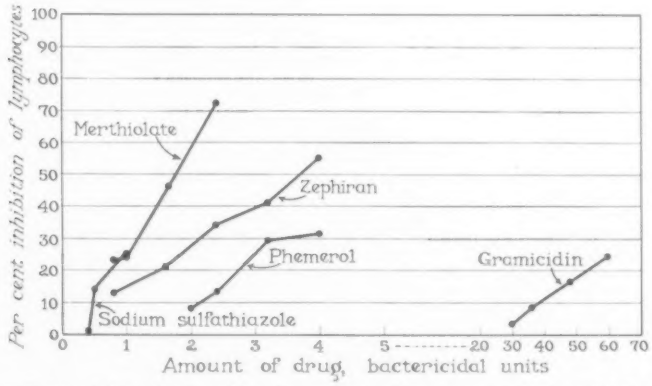

FIG. 2.—Cytotoxicity of germicides in concentrations expressed in multiples of the given drug necessary to inhibit *D. pneumoniæ*.

Figures 2, 3 and 4 represent the result of the combination of data given in Table 1 and Figure 1. Figure 2 represents the order of cytotoxicity of the germicides studied, with the exception of penicillin, in terms of the amount of drug necessary to inhibit pneumococci. The amount of drug used in each cytotoxicity test is expressed as the ratio of the actual amount of drug used in the test to the amount bactericidal for *D. pneumoniæ*. Thus, if the amount used in a toxicity test was

80 μg. per cc. and 20 μg. per cc. of the germicide was bactericidal for the strain of pneumococcus used, then the value was expressed as 4 bactericidal units in Figure 2. Figure 3 presents the toxicity of the germicides studied in relation to the amount of each one necessary to inhibit *S. aureus*, and Figure 4 is a similar representation in relation to the amount of each agent necessary to inhibit *S. pyogenes*. The toxicity of penicillin was so low compared with its bactericidal activity that the values obtained could not be included conveniently in Figures 2, 3 and 4.

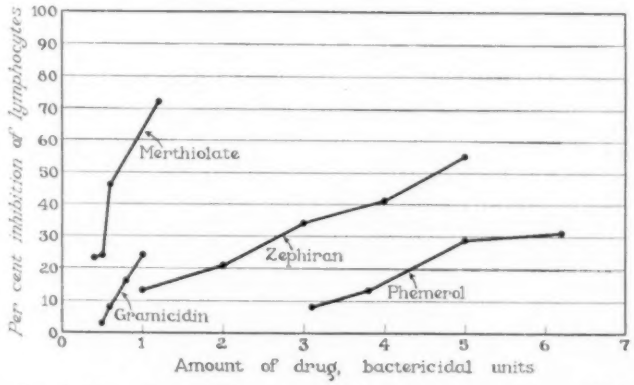

FIG. 3.—Cytotoxicity of germicides in concentrations expressed in multiples of the amount of the given drug necessary to inhibit *S. aureus*.

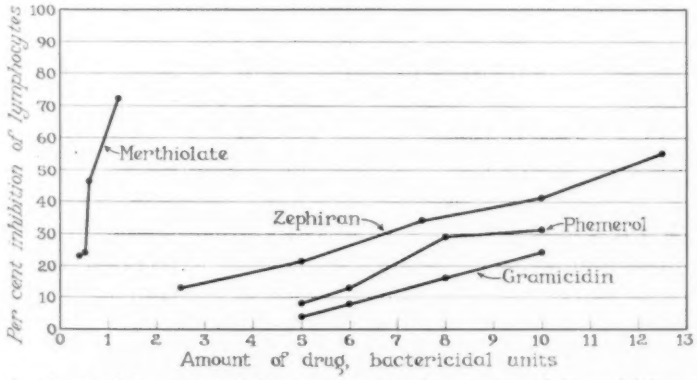

FIG. 4.—Cytotoxicity of germicides in concentrations expressed in multiples of the amount of the given drug necessary to inhibit *S. pyogenes*.

Baker, Harrison and Miller[1] have shown that the cationic detergents are frequently more bactericidal than the anionic detergents and that they are effective against Gram-positive and Gram-negative species. Zephiran and phemerol are cationic detergents and are effective in

small amounts against the Gram-positive cocci tested. They, like some other detergents, are hemolytic in small concentrations in the presence of serum and therefore could not be used with safety by the intravenous route.

We have previously pointed out[3] some of the biologic similarities between gramicidin and the anionic detergents. Baker, Harrison and Miller[2] have shown that the ability of gramicidin to decrease the respiration of bacteria is inhibited by the addition of demal, a unionized detergent capable of inhibiting the activity of anionic and cationic detergents alike. In accord with this observation, we have found that a bactericidal amount of gramicidin is rendered ineffective against pneumococci growing in the tissue culture clot if it is first mixed with a bactericidal amount of the cationic detergent, phemerol.[4] Bacteriostasis results only when an excess of either phemerol or gramicidin is present. These findings contraindicate the simultaneous use of gramicidin and a cationic detergent but do not necessarily contraindicate the use of gramicidin in the presence of the anionic synthetic detergents or any of the ordinary soaps. The antibacterial activity of penicillin was not inhibited by either gramicidin or the two cationic detergents, phemerol or zephiran.

Conclusions. A comparison has been made of the bactericidal and cytotoxic activity of gramicidin, penicillin and a few of the commonly used antiseptics. For the three organisms tested, namely, one strain each of *D. pneumoniæ*, *S. aureus* and *S. pyogenes*, penicillin was superior in germicidal properties to the other agents.

While gramicidin is effective in small amounts against *D. pneumoniæ* and *S. pyogenes*, it is relatively ineffective against *S. aureus*. On the other hand, zephiran and phemerol are quite effective against *S. aureus* as well as against the other two test organisms. Furthermore, penicillin is superior to the other germicides tested because of its low toxicity for tissues. Gramicidin produces less toxicity for tissue than the other germicides studied with the exception of penicillin. Of the other germicides, the two detergents, phemerol and zephiran, compare quite favorably with gramicidin in regard to their cytotoxicity.

The antibacterial activity of gramicidin, which behaves like an anionic detergent, can be neutralized by a cationic detergent, phemerol. The simultaneous use of gramicidin and a cationic detergent in the treatment of infections is, therefore, contraindicated. There is no contraindication to the simultaneous use of gramicidin with penicillin or the anionic detergents including the ordinary soaps.

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PROTRUDED INTERVERTEBRAL DISK AND HYPERTROPHIED LIGAMENTUM FLAVUM. CRITERIA FOR DIAGNOSIS AND INDICATIONS FOR OPERATION

WITH ANALYSIS OF 50 SURGICALLY TREATED CASES*

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THE concepts regarding the mechanism and management of low-back pain and the so-called sciaticas have in the past several decades reflected the advances of accurate knowledge as well as fads in medical practice. The gynecologic surgeon at one time sacrificed many normal organs in a futile attempt to relieve these symptoms. Suspension of viscera, especially the kidney, had its flare. Then followed the universally accepted concepts of sacro-iliac and lumbosacral sprains, the myositis of the Scandinavians, the fibrositis and fasciitis of the British and the focal infections of the Americans. All these concepts served some purpose and most of the patients apparently improved or recovered even though scientific proof of the validity of the concepts was frequently missing. In more recent years the deficiency states have received their share of attention and in the last 2 or 3 years the protruded intervertebral disk has become most prominent.

The clinicians for years have sought anatomic explanations for the puzzling problem of the low-back pain and sciatica. Carnett and Bates[2] long championed the concept of intercostal neuralgia, feeling that the nerve irritation was mechanical in nature and in the proximity of the intervertebral foraminæ. Feiling,[6] based on his studies of a large number of cases, felt that many of the so-called sciaticas are really forms of radiculitis. With a better knowledge of the specific inflammatory lesions of the roots as in tabes dorsalis, and in compressive lesions as in extramedullary spinal cord conditions, a better understanding of root irritation was obtained. It is therefore surprising that lesions of the intervertebral disks should have received so little attention until recent years.

The intervertebral disks make up about one-quarter of the entire length of the vertebral column and in the lumbar region are relatively larger, constituting about one-third of that part of the spinal column. Considering the anatomic makeup of the disks and the rôle they play in movements of the lower spine, it is not surprising that they are often the seat of disease or injury. Although attention was called to disease and injury of the disks as far back as 1896[7] and although cases of protrusion have been described as chondromas by many authors,[9] it was not until 1934 that Mixter and Barr[9] established the protrusion of the

* Presented as a part of a symposium on the protrusion of intervertebral disk, before the Orthopedic Club of Philadelphia, on November 12, 1942.

disk as a definite clinical entity. Since then it has become progressively more popular, reaching the point where some neurosurgeons[3] regard sciaticas and recurrent low-back pain as being almost universally due to a protruded disk.

Based on our experience we feel that the recognition of the protruded disk is an important practical addition in the management of the troublesome low-back pain and sciaticas. However, we feel that it is by no means the only important cause of this syndrome and that there is definite need for conservatism in diagnosis and management. It is with this in mind that we are reviewing 50 verified cases, each of which was personally studied by one of the authors and many by both. An attempt will be made to establish some criteria for diagnosis and treatment, and more particularly point out some pitfalls in the management of these cases. No attempt is made to review the literature, since this has been magnificently covered by Bradford and Spurling.[1]

Anatomy. The *gross* pathologic changes as observed at operation consist of one or more of the following:

1. Lateral protrusion of the nucleus pulposus with or without rupture of the annulus fibrosus are the most constant findings. The rather strong firmly attached posterior longitudinal ligament favors the lateral rather than the median protrusion. The protrusion is usually unilateral but may be bilateral. The lateral protrusion causes pressure on the spinal roots and explains the so-called "sciatic" pain.

The gross appearance of the excised disk depends on whether or not it was removed *en masse* or in several fragments. Removed in one piece, it measures from 0.5 to several centimeters and appears to consist of fibrous tissue which makes it look like wet, rolled up blotting paper. In addition there are often small, soft, pointed processes with a shiny surface. When it is removed in fragments, the appearance is less striking. The excised material is grayish white and, while usually soft, may be quite firm.

2. The lateral herniations are often productive of adhesions if they have ruptured through the annulus fibrosus. These adhesions may occur between the nucleus and root, or between the root and the ligamentum flavum. The density of the adhesions increases with the longevity and severity of the symptoms. In a few cases where the root sheaths were opened, dense adhesions were found within, matting together the fibrils and these in turn to the meninges. This may explain the residual symptoms in some of the cases.

3. Small herniations in the midline without rupture of the annulus fibrosus with little or no root compression were encountered in some cases. These cases were associated with considerable backache and but little pain in the lower limbs.

4. Hypertrophy of the ligamentum flavum associated with a herniated nucleus pulposus is an almost constant finding. The only cases where it was conspicuously not hypertrophied were the acute cases. The hypertrophy of the yellow ligament seemed to bear a relationship in degree to the length and severity of the symptoms, particularly those involving the back and its musculature.

5. Varices of the meningo-rachidian veins and of the root sheaths were found in a number of cases.

The *microscopic* changes in the protruded disk have been described by Deucher and Love.[4] In most cases there were microscopic changes in both the annulus fibrosus and nucleus pulposus. Fissures in the annular and nuclear parts were a constant finding. In the 100 cases studied, 32 showed degenerative changes, 28 fibrosis, and 22 edema.

The microscopic changes in the hypertrophied ligamentum flavum were described by Dockerty and Love,[5] and by Lewy and Groff.[8] The principal changes include splitting, fraying and fibrillation of the normal elastic fibers, varying degrees of fibrosis, hyaline changes in blood-vessels and occasional calcification.

Analysis of Surgically Treated Cases. The authors have observed many more than 50 cases, but 50 were selected because they were carefully studied and observed between 1938 and 1942 by at least one of us. A number of verified recent cases were not included by reason of lack of knowledge of end-results. With one exception, all these patients were operated on at Graduate Hospital, most of them by Dr. F. C. Grant and Dr. R. A. Groff, and a few by Dr. E. O. Jeffreys. One case was studied in Philadelphia and operated on by Dr. S. N. Rowe in Pittsburgh.

The more important findings are summarized in Table 1. These will now be briefly discussed.

Sex and Age Incidence. The condition is considerably more common in the male than in the female. It may occur at any age but the average age is 39.6 years.

TABLE 1.—ANALYSIS OF DATA

Sex	M, 31; F, 19
Age	23–64 (average, 39.5)
Trauma	28 (severe, 7; slight, 21; none, 22)
Duration of symptoms	Short, 3 mos.; long, 29 yrs.; average, 4.3 yrs.
Intermissions	Occurred, 32 cases; absent, 18 cases
Aggravating factors	Bending, 42; coughing and sneezing, 41; lifting, 37; walking, 35; sitting, 29; lying, 25
Location of symptoms:	
Initial	Backache, 41; pain in limbs, 9
Prior to operation	Back, 47; L. lower limbs, 29; R. lower limbs, 20
Degree of disability	Major, 42; moderate, 7; slight, 1

History of Trauma. In more than half of the cases there is a history of some trauma, as a rule slight. Lifting, falling and twisting of the trunk are the most commonly recorded modes of injury. There were relatively few major traumas of the vertebral column, which could be regarded as antecedent factors for the protruded disk.

Duration of Symptoms—Frequency of Intermissions. The average duration symptoms was 4.3 years. Intermissions were observed in 32 cases (varying from days to many years). A careful history in some severe cases would elicit that during the so-called free interval the patient was extremely careful not to "strain" the back. In some cases severe attacks were precipitated by mere bending or light lifting. On the other hand, some patients did hard work for years without any ill-effects.

Location of Symptoms. The *initial* symptom in the majority of cases was located in the back, usually in the middle and paravertebral portions of the back. Rarely was it unilateral and only in 9 cases did it radiate down one limb. Later in the course of the process backache still remained a prominent feature; but pain in the legs occurred in 49 of the cases, usually being unilateral.

Character of Pain and Aggravating Factors. The pain is usually deep, unaccompanied by nerve or superficial tenderness, and always aggravated by one or more of the several factors such as bending, sneezing and coughing, lifting, etc. One has the impression after examining these patients that unlike spinal cord tumors or inflammatory lesions the pain is mechanical in nature.

Degree of Disability. Of the 50 cases, 42 had a major disability, *i. e.*, were unable to pursue their work and required sedatives and some narcotics. Those moderately disabled could get along but had some pain, especially backache.

It should again be noted that backache is nearly a constant symptom in displaced disk, but unless it becomes severe the patient does not regard it as a real disability.

Objective Abnormalities. Though each patient received a detailed somatic and neurologic examination, only the more frequently encountered abnormalities are recorded here. Fortunately, these observations require but a few minutes to elicit. An inspection of the back and of the two pelvic crests followed by palpation and testing the mobility of the spine gives most valuable information. The following findings were noted: diminished lordotic curve, 47; tilting of pelvis, 36; muscle spasm, 48; alteration of knee reflexes, 9; alteration of Achilles reflexes, 21; hypesthesia, 4; Lasègue sign positive, 42 (bilateral, 22; unilateral, 20).

Each of these signs are, of course found in conditions other than the protruded disk. However, the occurrence of most of them in the same patient plus the suggestive historical data, and the exclusion of other disease makes these findings extremely important in diagnosis.

It should be stressed that the Lasègue test behaves rather peculiarly in some of these conditions, in that testing the contralateral leg gives rise to the inability to extend the tested leg, but pain in the opposite side.

Again it is worth stressing that sciatic nerve tenderness, so frequently observed in neuritis, was rarely seen in this series.

In addition, there were neurologic abnormalities of relative infrequency: There was 1 case of atrophy of the thigh and there were 3 with weakness and atrophy of one foot. One case had definite sphincter disturbances.

We learned in routine testing that normally the muscles of the *left* big toe, especially the extensors, are definitely weaker than those of the right big toe.

During the last 2 years we have used some of the newer tests which may prove of some value. One of these consists in flexing the chin upon the sternum, and the other in compressing the jugular vein.[11,12] Both of these tests proved of no more than confirmatory value.

Contrast Medium Studies. In the beginning only lipiodol was used as a contrast medium. More recently only air has been used. With few rare exceptions the air myelogram is a reliable diagnostic procedure. Air studies are unsatisfactory in the examination of lesions between L–V and S–1 and often in the cervical swelling of the spinal cord. Of the 42 oil medium studies, 3 were negative (1 disk L3 and L4; 1 disk L4 and L5; 1 negative exploration). Of the 9 air studies, 2 had both oil and air (1 negative on Roentgen ray study had a disk at L4 and L5).

It cannot be too strongly stressed that the radiologist who attempts these studies must be conversant with the various phases of the problem in order to attain a necessary technique and correct interpretations.

TABLE 2.—OPERATIVE FINDINGS AND RESULTS

Operative findings:		
Protruded disks only		40
Hypertrophied ligamentum flavum		7
Protruded disks and hypert. lig. flavum		6
Herniation of annulus fibrosus		1
Negative exploration		2
Location of protruded disks:		
L4–L5		23
L5–S1		10
L3–L4		8
L2–L3		4
D12–L1		1
Multiple disks		5
Location of hypertrophied ligamentum flavum:		
L4–L5		7
L5–S1		4
L3–L4		1
L1–L2		2
Results:		
Recovered		36
Improved		6
Unimproved (causes of failure in the 8 cases)		8
Associated psychoneuroses	3	
Vascular disease of lower limbs	2	
Appearance of disk symptoms elsewhere	1	
Associated degenerative disease of spinal cord	1	
Cause unknown	1	

Operative Findings and Results (Table 2). The location of the disks is quite in keeping with the larger statistics published by others. The negative explorations are not a source of great concern since these patients were operated on early in the experience of the surgeons and free manipulation was not used to extrude the injured disks.

Some of the cases of hypertrophied ligamentum flavum represented patients who were disabled for only a short time and who were operated against the choice of the senior author of this paper.

The results appeared gratifying in 36 of the 50 cases. Generally speaking, those who suffered longest and hardest gave the most gratifying results. But even among these 36, while the pain radiating down the leg was relieved, some backache remained, though not as a prominent symptom. Dr. Grant points out that prior to the operation the patient has so much suffering that he does not mind the slight backache due to the poorly functioning back which the operation did not correct.

One should not be overly optimistic about these results, however, as not enough time has elapsed to draw final conclusions.

Of the 8 failures, some of the patients had associated disease which may account for the continuation of the symptoms. These undoubtedly represent errors in diagnosis and judgment. In some cases, perhaps, adhesions were too extensive or formed again after operation.

Comment. *Problems in Diagnosis.* The analysis of the 50 cases operated upon indicates that the protruded disk and hypertrophied ligamentum flavum present fairly uniform historical data and objective abnormalities. It occurs most commonly in the fourth decade with a history of some form of trauma in more than 50% of cases. Low-back pain and pain in one or both extremities was recorded in most cases. This pain is mechanical in character with fairly constant aggravating factors. The average duration was over 4 years and intermissions were observed in more than half of the cases. The most constant objective abnormalities include the diminution of the lordotic curve, tilting of the pelvis, muscle spasm, positive Lasègue sign and alterations of the reflexes.

The above formulation is fairly uniform and suggestive but not pathognomonic of the protruded disk and hypertrophied ligament. Indeed, many other conditions have similar clinical history and objective abnormalities; in the final analysis, the diagnosis depends to a great extent, on the exclusion of other conditions. Therefore, before arriving at the diagnosis of a herniated disk, especially before advising operation, the clinician should be mindful of and rule out the following conditions, which can be conveniently divided into:

Conditions to be Considered in Differential Diagnosis. *Orthopedic Conditions.* Spinal anomalies, spondylolisthesis, sacro-iliac disease, lumbosacral disease, inflammations and neoplasms of the vertebral column, disorders of the articular facets, infections of the soft tissues (myositis, fibrositis, fasciitis), spasms of the fascia lata and pyriformis muscles.

Primary Neurologic Conditions. Neuritis, radiculitis, ganglionitis (including herpes zoster), lesions of the cauda equina and of the spina cord, with special reference to specific infections and tumors.

Visceral Disease. Posterior peritoneal cavity and pelvis, especially lower genito-urinary tract, prostate and rectum.

Systemic Processes. Focal infections, deficiency states.

Psychalgias.

There are many pitfalls in diagnosis. The many and varied conditions which the protruded disk may simulate, requires the clinician to be conversant with the normal and abnormal structure and function of the various parts involved and a close familiarity with or at least consciousness of the many diagnostic problems. It is therefore surprising to learn that some clinicians regard the diagnosis of a protruded disk as a simple matter. To the authors it appears that the utter disregard of contrast medium studies prior to operation for the majority of cases is at present untenable.

Therapeutic Problems. It is evident that protruded disk has existed in most cases for a long time before the laminectomy and some patients must have got well without spectacular surgery. It is generally agreed by most neurosurgeons that the acute back condition, muscle spasm, tilting, and so on, is not a neurosurgical problem. Most of these cases recover with rest, traction, physical therapy, and so on. Most have no recurrences and some have mild recurrences which are a source of brief disability of varying degree.

Another consideration is the effectiveness of the laminectomy in relieving the disability produced by the protruded intervertebral disk. The analysis of the 50 cases indicates recovery in 36 cases, improvement in 6 cases and failure in 8 cases. These were operated on within the last $3\frac{1}{2}$ years and the end-result cannot yet be accurately estimated. It is possible that some cases, now apparently recovered, may have to be otherwise classified in the future. In this connection, the reports of other observers are of interest.

After considering these various factors, the authors feel that no case in which the diagnosis is reasonably certain, should be regarded as a desirable operative subject, unless (1) previous adequate orthopedic and other treatment was afforded for a reasonable time (3 to 6 months) without appreciable improvement in the disability; (2) the existence of moderate to major disability; (3) frequently recurrent brief major disability or recurrent moderate disability for considerable periods.

Summary. Fifty surgically treated cases of protruded intervertebral disk and hypertrophied ligamentum flavum were analyzed from the standpoint of history, objective abnormalities, special studies, operative findings and end-results. The problems of diagnosis, differential diagnosis and treatment were discussed.

Conclusion. Based on the experience with the 50 cases, the following criteria for diagnosis and indications for operations are suggested: (1) Exclusion of other definite causes; (2) suggestive history; (3) presence of at least some objective abnormalities; (4) previous adequate orthopedic and other treatment for a reasonable time (3 to 6 months) with no appreciable improvement in disability; (5) presence of a major disability; (6) lasting recurrent attacks of moderate disability or frequent attacks of major disability; (7) contrast medium studies prior to operation for the majority of cases.

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THE EFFECT OF GLUCOSE ADMINISTRATION IN DIABETIC ACIDOSIS

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Introduction. The observations to be described were carried out to determine the effects of the administration of glucose to patients in diabetic coma. Even before the discovery of insulin, certain fundamental principles were successfully employed in the treatment of this complication of diabetes. The first was that the rapid metabolic rate in acidosis should be reduced by rest in bed and the use of a diet low in calories. Second, a decrease in the total amount of carbohydrate taken was used as a means of reducing the hyperglycemia and thus sparing pancreatic function. Third, treatment of the accompanying shock by the administration of adequate amounts of salt solution was considered essential. The object of treatment was primarily to improve carbohydrate metabolism, since it was recognized that ketosis, acidosis and finally clinical diabetic coma resulted from the primary disturbance in carbohydrate utilization. The introduction of insulin brought about immediate improvement in the results of management of diabetic coma and thus confirmed the tenet that control of carbohydrate metabolism is the essence of successful treatment. Yet fatalities still occur, usually in patients in whom outstanding features in the course of treatment have been the rise in blood sugar level in spite of insulin or the use of glucose solution intravenously or by mouth. That diabetic coma is a condition of acute insulin deficiency, the primary treatment of which is the provision of insulin in sufficient quantities, needs little demonstration. The administration of glucose by mouth and by vein therefore seems unreasonable unless it can be shown that glucose thus given is promptly oxidized, thereby reducing the rate of fat metabolism and ketogenesis, or else that the glucose thus given is stored as glycogen. If glucose thus administered merely accumulates in the intercellular body fluid, the question also arises whether this accumulation is harmless or whether under the conditions of diabetic acidosis the steady rise in the concentration of glucose in blood and intercellular fluid is actually harmful by bringing about a still greater degree of insulin deficiency. Our observations were carried out in the George F. Baker Clinic of the New England Deaconess Hospital primarily for the purpose of determining whether or not glucose given by vein or by mouth during diabetic acidosis is promptly oxidized.*

* The calculations of the carbohydrate burned were made in the usual empirical manner from the respiratory quotient and the total oxygen consumption. The value for total oxygen consumption was reduced by 15%, which was assumed to be the proportion of oxygen used in the combustion of protein, unless the urinary nitrogen was determined.[3,10]

Case Studies. CASE 1 (19697) a laborer, developed diabetes in October 1940 at the age of 23 years and came for treatment in early acidosis December 2, 1940. No diabetic heredity known. Present weight 159 pounds dressed. His first urine specimen contained 6% sugar and 3+ diacetic acid. He immediately went to the metabolism room at 3:45 P.M. After he had rested 30 minutes, the respiratory helmet was put on. The results of determinations of oxygen consumption and respiratory quotient are shown in Table 1.

TABLE 1.—METABOLISM IN DIABETIC ACIDOSIS BEFORE AND AFTER INTAKE OF GLUCOSE

Case 1 (19697) Oxygen Consumption and Respiratory Quotient (R. Q.)

Period	Time, P.M.	R. Q.	O_2 per minute, cc.
1	4.30	0.632	370
2	4.40	.671	362
3	4.50	.700	349
	Average	.668	360 = 2429 cals. per 24 hrs. at R. Q. = 0.70
	50 gm. glucose, 5.10—5.35 P.M., intravenously		
4	5.55	.662	345
5	6.10	.680	330
6	6.25	.684	327
7	6.40	.666	333
8	6.55	.681	331

Predicted heat production (Harris-Benedict) was 1749 cals.
His actual heat production was thus 39.7% above prediction.

At the beginning of the test the blood sugar was 200 mg. and the CO_2 content of the venous blood was 25 volumes per cent. The value for total acetone bodies in the blood (including acetone, diacetic acid and beta oxybutyric acid) was 58 mg. per 100 cc.

It is obvious that in this case the respiratory quotient remained constantly at a level indicating little or no combustion of carbohydrate and that the administration of 50 gm. glucose intravenously did not raise the respiratory quotient during the hour and 45 minutes following the administration of glucose solution. No evidence of combustion of the glucose is present.

TABLE 2.—*Case 1 (19697)* METABOLISM FOLLOWING ADMINISTRATION OF INSULIN AND GLUCOSE

Period	Time, A.M.	R. Q.	O_2 per minute, cc.	CH combustion* per 15 min. gm.
	100 units crystalline insulin at 8:47 A.M.			
1	9.05	0.756	258	0.92 avg. for periods 1–3
2	9.15	.781	262	
3	9.25	.802	262	
	Average	.780	261 = 1795 cals. per 24 hrs. (R. Q. 0.78) = +2.6%	
	50 gm. glucose intravenously, 9.44–10.07 A.M.			
4	10.30	.871	266	2.36
5	10.45	.874	254	2.30
6	11.00	.895	266	2.77
7	11.15	.781	301	1.07
8	11.30	.694	320	0.00

* In calculation assumed 15% of O_2 consumption (avg. basal O_2) was due to protein combustion.

The contrast between the effect of glucose administration in acidosis and after recovery appears in the next observations. During the interval between his admission on December 2 in early acidosis and December 10 he received a diabetic diet containing carbohydrate 160 grams, protein 80 grams and fat 86

grams and an insulin dose that, during the days preceding the test on December 10, included 32 units of crystalline insulin and 36 units of protamine zinc insulin. On the morning of December 10 the metabolic observations in the subjoined table were made. One hundred units of crystalline insulin were given at 8:47 A.M. Then the respiratory quotient was determined during the fasting period. This was followed by the intravenous injection of 50 grams of glucose after which respiratory quotient measurements were continued for two hours.

It will be seen that the respiratory quotient promptly rose from 0.780 to a maximum of 0.895 at 11:00 A.M. Carbohydrate (CH) combustion per 15 minutes rose from a fasting level of 0.92 gm. to a maximum of 2.77 gm. It is evident then that this rather severe diabetic patient, when given an adequate supply of glucose and a large dose of insulin, rapidly increased carbohydrate combustion and at the maximum the rate per hour was approximately the same as in normal men subsequently cited.

The effect of hypoglycemia then appeared. In the period ending at 11:15 he began to sweat but did not seem uncomfortable. During the final 8th period he was sweating definitely, and the blood sugar, which had been 160 mg. fasting, was 50 mg. at 11:30 A.M. The striking feature is that with the onset of hypoglycemia the respiratory quotient rapidly fell to a level indicating cessation of carbohydrate combustion. He was then given orange juice and made a prompt recovery from the hypoglycemic reaction.

The important fact here is that with the onset of insulin hypoglycemia an extremely abrupt change in the type of metabolism may take place. One may conceive that with the fall in blood sugar further oxidation of carbohydrate is stopped by a reflex mechanism in order to conserve the glucose that is present and thus protect the highly sensitive tissues of the brain, which might otherwise suffer serious damage from prolonged hypoglycemia. Although the respiratory quotient of the brain of the depancreatized animal is 1.0,[2,6] indicating its ability to use carbohydrate without the presence of insulin, hypoglycemia caused by excessive insulin not only reduces the supply of glucose available in the blood but seems to affect the ability of the brain to utilize even that limited supply. In this case no acidosis was noted. The urine passed at the end of the experiment and during the rest of the day contained no diacetic acid, no doubt because the hypoglycemic reaction was terminated so quickly. It is generally agreed that long-continued or severe insulin hypoglycemia will result in loss of liver glycogen, although insulin administered to depancreatized animals characteristically increases glycogen deposition in the liver from a low to a normal amount. In brief periods of insulin hypoglycemia the liver glycogen may be high, as in Case 6884, age 25 years, with diabetes of 11.7 years' duration. A biopsy of the liver revealed a fat content of 10.5% and a glycogen content of 12.1%. The respiratory quotient determined 1 hour before the biopsy was 0.82, and the blood sugar was 33 mg. No symptoms of hypoglycemia had occurred, indicating that the reflex mechanism associated with typical symptoms of hypoglycemia had not been aroused. In summary, we may say that in Patient 19697 during acidosis, glucose administered was not oxidized. After recovery from coma and after treatment with diet and insulin had been carried out for some days, when an excess of insulin was given such that an insulin reaction resulted in spite of the provision of an excessive amount of glucose, the amount of carbohydrate combustion at its peak did not exceed the rate of 11 gm. per hour.

Case 2 (20239) developed diabetes in May 1941 at the age of 14 years. He came for treatment on June 16, 1941, in early diabetic coma. His urine contained 3.4% sugar and 3+ diacetic acid. Early Kussmaul respiration was present. He entered the metabolism laboratory at 4:55 P.M. and the respiration helmet was put in place at 5:25 P.M. for the first 3 periods. The blood sugar at 6:18 P.M. was 230 mg., the CO_2 content of the venous blood was 18 volumes per cent, and the acetone bodies were 70 mg. per 100 cc. Intravenous administration of 50 gm. of glucose was begun at 6:25 P.M. At 8:00 P.M. the blood sugar had risen to 370 mg., and the CO_2 content of the blood was 21 volumes per cent.

Following the administration of 30 units of crystalline insulin at 8:05 P.M., the blood sugar fell at 9:00 P.M. to 180 mg. and the CO_2 value of the blood rose to 23 volumes per cent. He then received an additional 8 units of insulin at 10:00 P.M., and the next morning his fasting blood sugar was 60 mg., the CO_2 content of the blood was 38 volumes per cent, and the respiratory quotient was 0.72. In Table 3 are shown the individual values for the respiratory quotient and oxygen consumption in the preliminary periods before the administration of glucose, then those in a series of three periods following the intravenous injection of glucose, and finally those in 3 periods following the injection of insulin. In these last 3 periods there was a gradual, slight rise in the respiratory quotient.

TABLE 3.—*Case 2 (20239)* OXYGEN CONSUMPTION AND RESPIRATORY QUOTIENT IN COMA BEFORE AND AFTER ADMINISTRATION OF GLUCOSE AND INSULIN

Period	Time, P.M.	R. Q.	O_2 per minute, cc.	CH Combustion per 15 min. (gm.)
1	5.40	0.678	216	
2	5.50	.673	215	
3	6.00	.672	222	
	Average	.674	218 = 1471 cals.	0.0
	Intravenous dextrose (50 gms.), 6:25–6:52 P.M.			
4	7.05	.661	208	0.0
5	7.20	.679	206	0.0
6	7.35	.688	207	0.0
	Average	.676	207	
	30 units crystalline insulin intravenously, 8:05 P.M.			
7	8.15	.672	222	0.0
8	8.30	.687	219	0.0
9	8.45	.712	220	0.0
	Average	.690	220	

His heat production, predicted from the Harris-Benedict formula for men, is 1240 cals., and predicted from Table 20 in Pub. 303A, Carnegie Institution of Washington, it is 1236 cals.; thus his heat production was 18.6% above prediction.

Intravenous administration of glucose in this boy was not followed by any significant change in respiratory quotient or any evidence that the glucose was utilized. A decline in blood acetone occurred without a rise in respiratory quotient. The total blood acetone values fell from 70 mg. at 6:00 P.M. to 58 mg. at 8:00 P.M., 44 mg. at 9:00 P.M. and 28 mg. when he was fasting the next morning. The administration of insulin, however, brought about a slight rise in respiratory quotient, which was immediate and was still evident 12 hours later. At 9:15 P.M., 1066 cc. of urine were voided, the first urine voided after the urine that was voided at the beginning of the observation at 4:00 P.M. This urine contained 3.3% sugar, a total excretion of 35.2 gm. The nitrogen present was 4.4 gm. The next specimen, voided at 10:15 P.M., showed only a trace of sugar. We may then assume that the 35 gm. of glucose represented the total loss of glucose following the injection of 50 gm. of glucose in the metabolism test. In such a case, with early diabetic coma, recovery would probably have occurred without the use of insulin, as was occasionally true in the years prior to the discovery of insulin. The first factor that was effective was rest in bed and a restriction of diet in total calories so that the high metabolic rate was steadily reduced. This one factor alone in the early stage of acidosis will reduce the amount of fat catabolism and so reduce the amount of ketone body formation. In this case, the oxygen consumption fell during the periods from 7:05 to 7:35 P.M. as compared with the first 3 periods. A simultaneous fall in blood acetone values occurred. With the use of insulin the usual rise in oxygen consumption after glucose was observed. In this case there is no evidence for believing that any of the glucose injected was oxidized.

During acidosis the energy requirements are greatly in excess of the normal rate, as shown by our observations on patients in diabetic coma whose metabolic rates have varied between 20% and 48% above normal. To meet this high caloric need, some fat oxidation undoubtedly occurs in the muscles themselves. However, a considerable fraction, probably from one-third to one-half, of the total needs from fat is obtained by a preliminary oxidation of fat in the liver to ketone bodies only. Neither acetic acid nor glucose is formed by this oxidation. These ketone bodies are, under ordinary circumstances, freely utilized for energy by the peripheral tissues without insulin. When the demand for calories from fat exceeds a level of approximately 2.5 gm. of fat per kg. body weight per day in a resting state, ketone bodies in excess of needs are formed by the liver.[13] If this excessive fat catabolism continues unchecked, ketosis and coma follow. That a fairly close parallel exists between the degree of acidosis and the degree of elevation of the metabolic rate was shown by Joslin.[7] In our patients, then, with acidosis we find an excessively high metabolic rate, the caloric needs for which are met almost entirely from fat and in these patients no improvement in the type of metabolism will occur until a sufficient quantity of insulin, endogenous or exogenous, is available to make carbohydrate combustion take its normal place. The metabolic rate of Case 20239 was 61 calories per hour. The nitrogen excretion, a total of 4.4 gm. in 9 hours, equalled 0.5 gm. per hour, which is equivalent to a protein metabolism of 3.1 gm. per hour. We assume 12 calories from protein and subtract this from 61 calories per hour, which leaves 49 calories to be derived from fat, which would mean a rate of 131 gm. per day. His weight was 35 kg., so that this would greatly exceed the rate of 2.5 gm. of fat per kg. body weight postulated by Stadie.[13] Effective insulin action in acidosis results in a reduction of the metabolic rate and in his case the fall in oxygen consumption was from +18.6% (June 16, 5:40 P.M.) to 3.2% above the standard as measured the following morning.

TABLE 4.—EFFECT OF INSULIN UPON CARBOHYDRATE COMBUSTION

Period	Time, P.M.	R. Q.	O_2 per minute, cc.	CH combustion per 15 min., gm.
1	1.10	0.709	299	
2	1.20	.701	309	
	Average	.705	304	0.00
	40 units crystalline insulin at 2:20 P.M.			
3	2.40	.713	296	0.00
4	2.55	.731	296	0.20
5	3.10	.717	305	0.02
	Rest period			
6	4.05	.731	287	0.17
7	4.20	.733	296	0.20
8	4.35	.727	295	0.07
	Rest period			
9	5.45	.723	292	0.04
10	6.00	.739	297	0.33
11	6.15	.727	304	0.10

CASE 3 (20945), school boy, age 15 years, demonstrates the effect of insulin alone upon the respiratory quotient during acidosis. He came for treatment on January 21, 1942, with diabetes which had just been discovered. Upon arrival the urine contained 8.7% sugar, 4+ diacetic acid and the CO_2 of the blood was 31 volumes per cent. He was sent to the hospital at noon. The respiration helmet was placed at 1:10 P.M. Forty units of crystalline insulin were given at 2:20 P.M. and a series of observations was carried on ending at 6:30 P.M. The blood sugar was 360 mg. on admission and 101 mg. at the end of the observation. The data are summarized in Table 4.

During the period of study a marked change in nitrogen excretion in the urine occurred as shown in Table 5:

TABLE 5.—*Case 3.* NITROGEN AND CREATININE ELIMINATION IN DIABETIC ACIDOSIS

Collection Period				Nitrogen eliminated			Creatinine eliminated		
Date 1942	Start	End	Vol., cc.	Per cc., mg.	Total, gm.	Per hour, mg.	Per cc., mg.	Total, mg.	Creat. coeffi., mg./kg./24°
Jan. 21	11.00 A.M.	1.45 P.M.	780	3.91	3.05	1109	0.46	361	67.4
" "	1.45 P.M.	3.45 P.M.	258	5.50	1.42	710	0.52	134	34.5
" "	3.45 P.M.	6.30 P.M.	100	10.63	1.06	387	1.58	158	29.4
Jan. 21, 6.30 P.M., to Jan. 22, 7.00 A.M.			520	8.14	4.23	339	0.90	470	19.3
Total for 24 hours			1990	5.88	11.71	488	0.68	1348	28.8

It is evident that during the period from 11:00 A.M. to 1:45 P.M. the nitrogen elimination per hour was at the rate of 1109.1 mg. During the next 2 hours the rate fell to 710 mg. and during the period from 3:45 P.M. to 6:30 P.M. the elimination fell to 387 mg. per hour. During the next 12 hours this rate fell to 339 mg. During these same periods a comparable change in creatinine elimination was observed with the result that the creatinine coefficient fell from 67.4 to 19.3 mg.

This case indicates that the use of insulin alone in a patient with early diabetic acidosis will increase carbohydrate consumption even when only a small dose, such as 40 units, is given. The fact is that only a small amount of increase in glucose oxidation is necessary. Heat production in this patient was at the rate of *85.5* calories per hour. Carbohydrate oxidation amounted to *0.47* gm. in 45 minutes of the last 3 periods. Thus approximately *2.5* calories per hour were obtained in the oxidation of glucose.

Rate of Oxidation of Glucose in Normal Subjects. The rate of the utilization of injected or ingested glucose in the normal individual is low; in the resting state only between 6 and 12 gm. per hour are oxidized. Therefore, in any consideration of the part played by the oxidation of glucose in diabetic patients normal standards must be considered. A normal male, 43 years of age, weight 66.8 kg., who ingested amounts of glucose varying from 5 to 104 gm. was studied by Carpenter and Fox.[4] Their results clearly show that in the majority of the observations the greatest rise in respiratory quotient indicating the greatest increase in carbohydrate combustion was observed during the third half hour following the ingestion of the glucose. All amounts of 10 gm. or more were followed by significant changes in the respiratory quotient. The striking fact, however, was that no close relationship was found between the amount of glucose ingested and the increase in grams of carbohydrate oxidized per hour. Thus, after the subject received 15 gm. of glucose, the carbohydrate metabolized during the first 2 hours was 14.25 gm. which represents an increase over the fasting rate of 3.17 gm. When he received 25 gm. of glucose, the amount of carbohydrate metabolized in the same first 2 hours was 16.2 gm., an increase of 4 gm. When he received 104 gm. of glucose, the carbohy-

drate metabolized during the same 2 hours totaled 18.8 gm., an increase of 10.3 gm. The facts regarding this normal man are so important that they are summarized in Table 6. It is evident that in the first $3\frac{1}{2}$ hours following the ingestion of 104 gm. of glucose, when his fasting or basal respiratory quotient was 0.81, the total carbohydrate metabolized was 35.4 gm. The same point appears in the studies of normal young men receiving 50 gm. of glucose by mouth and by vein, reported by Root and Carpenter.[11] The *increases* in carbohydrate combustion during $2\frac{1}{4}$ hours after the administration of 50 gm. of glucose averaged in 4 subjects 10.6 gm. by the oral route and 9 gm. when the glucose was given intravenously. Therefore, in our observations of diabetics, it is important to remember that even in normal individuals, no matter how much glucose is given by vein or by mouth, in a resting state only between 6 and 12 gm. per hour are oxidized. Obviously if extreme muscular exertion is being carried out, glucose oxidation will be increased.

TABLE 6.—OXIDATION OF GLUCOSE BY A NORMAL MAN[4]

	Carbohydrate Combustion (gm. per half hour)
Basal	2.13
Half hours after 104 gms. glucose	
1	3.33
2	5.31
3	5.20
4	5.01
5	4.85
6	6.16
7	5.58
Total	35.44

Total increase over basal 20.2 gm.

Harmfulness of Excessive Glucose During Diabetic Coma. Not only is the administration of large amounts of glucose useless and ineffective in diabetic acidosis, but such a procedure may be positively harmful in that (1) a rise in blood sugar produced by glucose will make it difficult to determine the required insulin dosages by changes in the blood sugar; (2) such excessive hyperglycemia is always harmful to the pancreas; (3) excessive glucose concentration in the blood and tissues under the conditions of acidosis has been known to result in anuria;[12] (4) the experiments of Astwood, Flynn and Krayer[1] indicate that excessive glucose may lead to damage in the liver. Recently a most valuable contribution to the study of this problem appeared in a case reported by Grunberg and Rhodes.[5] Their patient, a boy, age 15 years, had been under observation as an out-patient for 6 months with a blood sugar content varying from 120 to 240 mg. per 100 cc. He was admitted in diabetic coma at 12:30 P.M. with semi-consciousness, dry skin, soft eyeballs, blood pressure systolic 70, diastolic 50, blood sugar 425 mg., urine sugar 3%, and gross ketonuria. Table 7 summarizes the insulin dosage, blood sugar values, and glucose administration. The boy did not seem in far-advanced coma, as he was not entirely unconscious and was not pulseless. Furthermore, anuria was not present. The

extraordinary feature was that this case of coma should have advanced to such a condition at 1 A.M. the following morning that the authors felt that he was moribund. At 3 A.M. it was believed "that he would be dead in an hour or two." As we analyze the table, it is evident that he received 175 gm. glucose at the outset of his treatment when only semi-conscious and in spite of a dose of 215 units of insulin during that same period the blood sugar had risen to 600 mg. by 5 P.M. Thereafter the blood sugar continued to rise in spite of generous insulin dosage, until finally a sufficient insulin dose brought about recovery. It is instructive to make a calculation of the relation between the glucose administered by mouth and by vein and the grams of glucose present in the intercellular fluid,* as represented by the increase in blood sugar concentration from 425 to 1600 mg. The boy's weight was 36 kg. We may assume that the intercellular fluid was roughly 25% of his weight or about 9 liters. An increase in blood sugar of 1175 mg. per 100 cc., when applied to this volume of intercellular fluid, would indicate that there had been a total increase in glucose in this fluid of 105 gm. This increase in glucose alone would greatly increase the requirement of insulin. If we return to the figures for diabetic patients and for normals, we find that the most one can hope for is combustion of from 5 to 10 gm. of glucose in the first 2 hours of the emergency in diabetic coma. The administration of more glucose when the blood and intercellular fluid already contain glucose in excessive amounts can have no possible benefit, unless there is a previous administration of insulin in such amounts as will surely compel glucose utilization. This boy strikingly illustrates the fact that recovery in diabetic coma occurs when carbohydrate utilization takes place at such a rate that the concentration of glucose in the blood and intercellular fluid falls rather than rises. The administration of excessive amounts of glucose imposes such a burden on the insulin injected as to retard and impede its action. In the last series† of 73 successive coma cases with blood CO_2 of 20 volumes per cent or less, at the New England Deaconess Hospital reported by Joslin, Root, White and Marble,[8] no fatalities have resulted, and we still feel that although the absence of complications fatal in themselves in this group of cases cannot be ignored, emphasis upon the rapid administration of insulin in doses sufficient to bring about utilization of the excessive glucose already present in the blood as indicated by a fall in the blood sugar within the first 5 or 6 hours, the administration of sufficient fluid in

* An increase of blood sugar from 425 to 1600 mg/100 cc., means an increase of 65 millimols per liter of fluid. If this is superimposed upon the usual osmolar concentration of body fluids of 320 mM/L, that would mean an osmolar concentration of 385 mM/L, or a markedly hypertonic solution, and a consequent shrinking of cells generally, unless the *intra*cellular fluids of the tissues become hypertonic at the same time to the same degree. If one were to assume that the glucose was uniformly distributed through the water of the tissue, both intracellular and extracellular, one might figure that he had 293 gm. more sugar in his body fluids at 1:10 A.M. than at 12:30 P.M. This is assuming that there was 25 kg. of H_2O in a 36 kg. boy, and all the water had increased its sugar content 11.75 gm. per kg. Whether glucose is distributed thus uniformly through the tissues of the body is not yet known.

† In the original report the number of successive cases of diabetic coma without fatality was 62, but has since risen to 73.

the form of physiologic salt solution and the inauguration of hourly feeding from the 6th hour onward were the essential factors in the treatment.

TABLE 7.—EXTREME HYPERGLYCEMIA FOLLOWING ADMINISTRATION OF GLUCOSE IN DIABETIC COMA, BOY, 15 YRS.[5]

Time	Blood sugar, mg.	Glucose (grams)		Insulin (units)		Clinical condition
		By mouth	Intravenously	Subcutaneous	Intravenously	
12.30 P.M.	425	...	..	...	...	Semi-conscious
1–5 P.M.	600	150	25	140	75	
5–8.40 P.M.	1040	20	..	100	300	Unconscious
9–11.30 P.M.	920	...	..	...	800	
1.10 A.M.	1600	...	..	...	...	Moribund
1.30–3 A.M.	...	...	..	...	800	
9.00 A.M.	160	...	..	...	...	Better
11.00 A.M.	65	40				
12.30 P.M.	112					
1.30 P.M.	...	...	..	...	...	Almost conscious
2.10 P.M.	180	160				

Causes of Diabetic Acidosis. Undoubtedly deprivation of insulin is the most frequent factor precipitating diabetic coma, but also it must not be forgotten that deprivation of insulin may mean not the omission of a dose of insulin but merely that the patient's own supply of endogenous insulin has become inadequate for increased metabolic needs, as in cases of diabetic coma developing before the diagnosis of diabetes has been made. Or a constant supply of insulin may become inadequate by virtue of a great increase in the total metabolism or by influences such as infection or exhaustion or hyperglycemia with the associated changes in the tissues that impair glycogen storage. There can be no doubt that in some mild diabetic patients the addition of carbohydrate may stimulate insulin production and increase in a limited way the amount of carbohydrate retained. There is no evidence that this is the case in serious cases of diabetic acidosis. Any complication that accelerates the loss of glycogen in the liver, such as hyperthyroidism, endocrine disturbances, infection, anesthesia, nausea, vomiting, will contribute to severity and rapid development of diabetic coma. However, glycogen starvation of the liver without diabetes, as it is observed in fasting or intense hyperthyroidism, never produces a condition resembling diabetic coma. It is only when insulin deficiency is present that glycogen impoverishment in the liver becomes an important element in the clinical state. It is too commonly forgotten that the administration of glucose in large amounts producing hyperglycemia has an exhausting effect upon the islands of Langerhans,[9] still further diminishing their insulin-producing function, and, furthermore, that it is possible with excessive administration of glucose in animals, as shown by Astwood, Flynn and Krayer[1] at the Peter Bent Brigham Hospital, to produce actual destruction of liver tissue.

Summary. 1. In diabetic coma the administration of glucose solution either intravenously or by mouth does not result in an increase in respiratory quotient indicating any increase in carbohydrate combustion.

2. Insulin produces an increase in carbohydrate combustion, indicated by a rise in respiratory quotient.

3. Even with insulin administration there is no evidence that more than 10 gm. of carbohydrate can be or need be oxidized per hour, in order to reduce the rate of fat metabolism and so to check the process of ketone body formation.

4. The harmful effect of glucose may be concealed in early diabetic coma by the favorable effects of insulin simultaneously administered; the moderate case of coma may be converted into a severe one requiring excessive insulin dosage. In advanced coma, glucose administration may precipitate the final stage of anuria.

5. The object of treatment in diabetic coma is to restore normal utilization of carbohydrate by the administration of the requisite amount of insulin. By this means, the excessive glucose in blood and tissue fluids is oxidized or stored, liver glycogen is replenished and excessive ketosis is reduced by a reduction in the rate both of total metabolism and of fat oxidation.

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CORRELATION OF THE INTRAVENOUS HIPPURIC ACID TEST OF LIVER FUNCTION WITH BODY SIZE

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The excretion of hippuric acid after the administration of sodium benzoate, formerly intended as a measure of renal function,[3,6] has been studied extensively by Quick.[8,9] In 1933 he proposed it as a test of liver function.[10] After some delay this test has received increasing attention.

Quick showed[8] that, regardless of the amount of sodium benzoate given, beyond a certain minimal quantity, and in the absence of exogenous glycine, a relatively constant amount of hippuric acid will be excreted in the urine. With an adequate dose of sodium benzoate, the amount of hippuric acid formed depends primarily upon the capacity for synthesis of glycine, presumably in the liver, and also upon the capacity to conjugate it with benzoic acid.

Quick[8] stated that the rate of synthesis of glycine appeared to be a constant, depending on the size of the individual. He later[11] stated that the rate of excretion of hippuric acid is influenced only somewhat by the size of the subject. As it was planned,[10] a maximal load was supposed to be placed on the liver by the hippuric acid test. Presumably every cell is forced to its greatest capacity in the synthesis of glycine for conjugation. It seemed, therefore, that the rate of formation of hippuric acid should vary normally with the size of the liver. If this is so, it seemed that a variable standard of normal hippuric acid excretion, based upon the size of the subject, would be more accurate than a single constant standard.

There are no adequate data available to indicate which body measurement—weight, height ("normal" weight), or surface area—would be the most accurate basis for prediction of normal liver size and consequently the expected excretion of hippuric acid. We have calculated from the data of Greenwood and Brown[5] of 78 cases with "normal organs" that those having body weights within 25 pounds of the predicted normal (most subjects were below normal weight) had livers averaging 35.6 ounces per square meter of surface area or 0.433 ounce per pound. Those who were more than 25 pounds underweight had livers averaging 32.7 ounces per square meter or 0.455 ounce per pound. There was, however, such a very wide range in the ratios of liver weight to body size that these figures are not significant.

Boyd,[2] in a statistical study of 312 cases dying within 24 hours of accident or similar causes without gross hemorrhage and without other significant disease, reported that the liver is essentially stationary in weight from 20 to 55 years and then decreases in weight. The possible significance of this will be referred to later although correlation with body size was not made. Bean and Baker[1] reported that the liver of the negro is smaller than that of the white man.

Because of a frequency of nausea (and sometimes vomiting), greater than has commonly been reported, among our first series of normals to whom sodium benzoate was given orally, the intravenous route more recently proposed by Quick, Ottenstein and Weltcheck[12] was used for subsequent tests. The results obtained with this type of test dose are the basis of this report.

Method. The tests were done in the morning after a breakfast of toast and coffee, as advised by Quick.[10] Sodium benzoate (1.77 gm. in 20 ml. of water) was injected intravenously over a period of 5 minutes and urine specimens were collected 1 hour after the completion of the injection as recommended by Quick, Ottenstein and Weltcheck.[12] Hippuric acid was determined by the simpler clinical method of Quick,[10] except that ammonium sulfate was added before precipitating the acid as he subsequently advised.[13]

TABLE 1.—DATA ON THE INTRAVENOUS HIPPURIC ACID TEST ON SUBJECTS UNDER 55 YEARS OF AGE

Case	Diagnosis	Sex	Age (yrs.)	Ht. (in.)	Wt. (lbs.)	Surface area (sq. m.)	Hippuric acid excreted (gm.)	Predicted excretion (surface area)	% of predicted excretion (surface area)	Predicted excretion (lbs.)	% of predicted excretion (lbs.)
1	Student	M	24	76¾	202.0	2.21	1.36 1.48 1.42 Av.	1.69	84.1	1.69	84.1
2	Student	M	21	73.0	195.5	2.11	1.64	1.60	102.4	1.64	100.0
3	Instructor	M	31	74.5	180.0	2.08	1.73 1.48 1.60 Av.				
4	Student	M	24	76.5	166.0	2.02	1.66 1.53 1.60 Av.	1.585	100.9	1.54	104.0
5	Duodenal ulcer	M	46	71.0	185.0	2.02	1.37	1.523	90.0	1.58	86.7
6	Instructor	M	27	73.5	160.0	1.95	1.33	1.47	90.5	1.41	94.3
7	Student	M	24	72.0	160.0	1.92	1.31 1.28 1.30 Av.	1.445	89.9	1.41	92.2
8	Student	M	24	70.5	161.0	1.88	1.57	1.41	111.3	1.415	111.0
9	None	M	34	70.0	159.0	1.88	1.25	1.41	88.6	1.40	89.3
10	Student	M	22	72.0	155.0	1.90	1.45	1.43	101.3	1.375	105.5
11	Student	M	23	70.5	160.0	1.89	1.67	1.42	117.7	1.41	118.4
12	Student	M	25	70.5	159.0	1.87	1.41	1.40	99.2	1.40	100.7
13	Student	M	23	68.5	157.0	1.84	1.53	1.38	110.8	1.39	110.0
14	Duodenal ulcer	M	30	70.0	148.5	1.82	1.31	1.36	96.4	1.33	98.5
15	None	M	42	69.0	145.0	1.78	1.34	1.33	100.7	1.31	102.2
16	Psychoneurosis	F	32	66.0	153.0	1.76	1.40	1.31	106.9	1.36	102.9
17	C.N.S. disease	M	22	68.0	142.0	1.75	1.32	1.31	100.6	1.29	102.3
18	Uterine fibroid	F	35	65.0	145.0	1.71	1.59	1.27	125.3	1.31	121.3
19	Compound fracture	M	16	66.0	141.0	1.71	1.20	1.27	94.5	1.28	93.7
20	Asthma	M	16	67.0	134.0	1.69	1.33	1.252	106.2	1.236	107.6
21	Student	M	23	66.5	132.0	1.67	1.59 1.33 1.46 Av.	1.235	118.2	1.22	119.7
22	None	F	29	62.0	149.0	1.67	0.82	1.235	64.4	1.335	61.4
23	Chronic pelvic disease	F	29	63.0	134.0	1.67	0.90	1.235	72.8	1.236	72.8
24	Lues—liver neg. at postmortem	M	53	66.5	130.0	1.66	1.22	1.223	99.7	1.21	100.8
25	Allergic bronchitis	F	30	64.0	124.0	1.66	1.42	1.225	115.8	1.17	121.3
26	Mild obesity	F	40	62.0	145.0	1.65	1.73	1.22	141.7	1.31	132.0
27	Duodenal ulcer	M	38	64.5	134.0	1.64	1.32	1.21	109.0	1.236	106.8
28	Duodenal ulcer	M	40	66.0	130.0	1.64	1.10	1.21	90.0	1.21	90.9
29	Dietitian	F	25	66.5	127.0	1.64	0.94	1.21	78.0	1.19	79.0
30	Nurse	F	20	63.5	133.0	1.62	1.44	1.195	120.0	1.23	117.0
31	Dietitian	F	25	65.0	127.0	1.62	1.11	1.195	93.0	1.19	93.3
32	Mild pelvic inflam. dis.	F	21	67.0	119.0	1.60	1.00	1.177	84.9	1.135	88.1
33	Student	M	26	64.0	123.0	1.57	1.11	1.15	96.5	1.16	95.7
34	Technician	F	28	65.0	118.0	1.57	1.30	1.15	112.7	1.13	115.0
35	Arthritis	F	39	64.0	121.0	1.57	0.94	1.15	81.7	1.15	81.7
36	Subarachnoid hemorr.	F	22	61.0	129.0	1.55	1.29	1.135	113.7	1.20	107.5
37	Technician	F	23	63.5	121.0	1.55	1.49	1.135	131.0	1.15	132.3
38	Clinic clerk	F	30	64.0	117.5	1.54	1.34	1.123	119.3	1.125	119.2
39	Bronchiectasis	F	18	65.0	106.5	1.50	0.74 0.74 0.74 Av.	1.090	67.9	1.05	70.5
40	Clinic clerk	F	27	60.0	117.0	1.47	1.07	1.065	100.5	1.12	95.5
41	Nurse	F	22	61.0	111.0	1.46	1.00	1.06	94.4	1.08	92.6
42	Anxiety	F	15	62.0	102.0	1.43	1.07	1.033	103.6	1.025	104.3
43	Pneumonitis	F	18	62.0	97.0	1.38	1.01	0.99	101.9	0.99	102.0
44	Subacute rheum. fever	F	23	63.0	87.0	1.34	0.65	0.96	67.7	0.92	70.7
45	Clinic clerk	F	27	62.0	89.0	1.34	0.89	0.96	93.1	0.93	95.8
46	Anxiety	F	28	63.0	86.0	1.33	0.90	0.95	94.7	0.91	99.0

Results. The intravenous hippuric acid test was performed upon 46 subjects, mostly young adults; 23 were males and 23 females. Twenty-two subjects were students or staff members in presumably good health and 24 were patients without apparent liver disease. The excretion in these subjects varied from 0.65 to 1.75 gm. of hippuric acid in 1 hour (Table 1). We have preferred to dispense with the conversion of these figures into their equivalents of benzoic acid.

The results of these tests have been studied statistically. No difference in the excretion for the sexes was found. The amount of hippuric acid excreted was compared with the body weight, height, ideal weight as obtained from insurance tables, and surface area. A definitely significant correlation between the hippuric acid excretion and both body weight and surface area was found, the correlation being almost identical for the two factors.

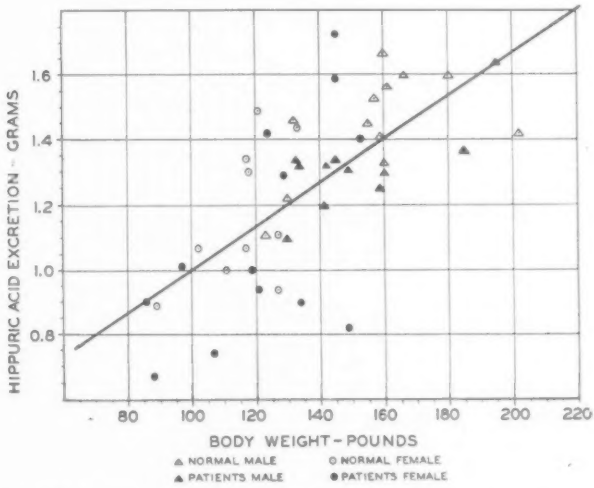

CHART 1.—The excretion of hippuric acid in 1 hour after the intravenous injection of 1.77 gm. of sodium benzoate by 46 normal subjects and patients without liver disease under 55 years of age.

The correlation coefficient for the weight of the subjects compared with the grams of hippuric acid excreted was 0.674, with a standard error of 0.193. The predicted excretion based upon the weight of the subject is expressed by the equation: Hippuric acid (grams) = 0.34 + (0.00668 × weight in pounds). This straight line formula and the data upon which it is based are shown graphically in Chart 1.

The correlation coefficient for the surface area of the subjects compared with the grams of hippuric acid excreted is 0.668, with a standard error of 0.194 gm. The equation for the predicted hippuric acid excretion on the basis of surface area is: Hippuric acid (grams) = (0.837 × surface area in square meters) − 0.163.

Comment. These findings indicate that the excretion of hippuric acid in this test normally varies with the size of the individual. Nearly

all of the subjects used were of about normal weight. It is possible that with a sufficient number of tests upon subjects with a wider range of body build, a preference would be established for the use of body weight or surface area as a basis for normal standards.

Of the normal subjects, 90% excreted between 72.8% and 125% of the predicted normal amounts of hippuric acid. This is a rather wide range of apparent hepatic function. The range of normal values based upon these determinations would, however, need to be much larger if the normal standard were not based upon body size. The 5 largest subjects excreted an average of 1.53 gm. of hippuric acid while the 5 smallest subjects excreted an average of only 0.9 gm. Although 3 subjects excreted somewhat less than 70% of the normal amount of hippuric acid predicted on the basis of surface area and 1 subject, less than 70% of that predicted on the basis of body weight, we would consider that figure (70%) the borderline of normal excretion with this test in subjects between the ages of 20 and 55 years.

TABLE 2.—HIPPURIC ACID EXCRETION IN PATIENTS OVER 55 YEARS WITHOUT KNOWN LIVER DISEASE

Diagnosis	Age	Sex	Height (in.)	Weight (lbs.)	Ideal weight (lbs.)	Hippuric acid	
						Excreted	% of predicted excretion
Hypertension	58	M	68	142	154	0.95	74
Cancer of larynx	70	M	69	139	158	0.92	73
Nucleus pulposus	56	M	71	159	168	1.13	80
Cancer of lip	64	M	71	184	168	1.04	64
Cord tumor	60	F	63	186	128	0.67	41
Peptic ulcer	64	M	..	120	..	1.01	90

Tests were done on 6 additional patients of 56 years or over who had no apparent evidence of liver or kidney disease. They excreted an average of 70.3% of the amount of hippuric acid predicted from their surface area (Table 2). This suggests that in older age groups there is physiologically some atrophy of the liver which causes some impairment in the response to the hippuric acid test of liver function. This is in accord with the findings of Boyd[2] although, as was noted, the liver weights in her series were not correlated with body size. The effect on her figures of the influence of relative weight on longevity is consequently not known.

No serious complications resulted from the slow intravenous injection of sodium benzoate. Many patients have noted an aching sensation along the course of the vein, particularly when the injection was given more rapidly. Two subjects, among this group and other patients on whom the test was done, have had sudden severe abdominal cramps sufficient to cause interruption of the test. In one allergic individual, a moderately severe urticaria developed immediately after the injection and lasted about 1 hour. We have also seen urticaria appear after the oral administration of sodium benzoate and have noted the occurrence of allergic types of reactions following the intravenous injection of bromsulphthalein.

The chief advantage of the intravenous hippuric acid test is that it avoids the nausea and vomiting which occurred frequently in our normal subjects. This has been particularly helpful in performing the

tests on patients who already have abdominal symptoms. The intravenous administration also eliminates the factor of absorption from the gastro-intestinal tract and shortens the test.

The possible disadvantage of the intravenous method is the greater effect on the result of inaccuracy in collecting completely a 1 hour specimen rather than a 4 hour specimen of urine. It is probable that some of the variability of results in this series was due to incomplete emptying of the bladder. This source of error may be minimized by provision for an adequate urine volume. Several of our patients, not in this series, with 1 hour urine volumes of 10 to 15 cc. had small excretions of hippuric acid which subsequently were normal when the preliminary fluid intake was increased. The test is, of course, of no value when there is significant urinary bladder retention unless the bladder is catheterized.

The value of the hippuric acid test of liver function presupposes an adequate renal function. Snapper and Grünbaum[14] have been quoted that the excretion of hippuric acid was normal in cases without retention of urea. Their series was small and, with 12 hour urine specimens, delays in excretion could have been overlooked. Kohlstaedt and Helmer[7] found a subnormal excretion of hippuric acid in 9 subjects with urea clearances of 50% or less, 4 of whom had no evidence of liver disease. Quick[8] has shown that there was more than a 50% increase in hippuric acid excretion when glycine was taken with the sodium benzoate, indicating at least that much greater normal capacity for renal excretion over the synthesis of hippuric acid.

Campbell[4] has shown that patients who have been depleted of glycogen postoperatively have decreased excretions of hippuric acid which quickly return to normal following restoration of carbohydrate.

Summary. The excretion of hippuric acid following the intravenous injection of sodium benzoate as a test of liver function in 46 subjects without apparent liver disease has been found to be influenced decidedly by the size of the subjects.

Formulæ for the predicted normal excretion of hippuric acid, based on body weight and on surface area, are presented.

The correlation of hippuric acid excretion with these two measures of body size was almost identical in this series of tests.

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A COMPARISON OF TECHNIQUES FOR THE DIFFERENTIAL COUNTING OF BONE MARROW CELLS (GUINEA PIG)

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The variations in technique used by different workers in obtaining and preparing specimens of bone marrow add considerably to the uncertainties which feature in any evaluation of differential counts of marrow. The techniques which have been employed in the preparation of specimens for counting may be classified into one of three general methods, namely, the use of fixed smears, of sections, and of supravital preparations.

The present work was planned for the purpose of comparison of the counts made by these three general methods when employed by the same individuals, and applied to the same marrow. No technique was found for the preparation of fixed smears, however, which yielded as representative a distribution of the cells, caused as little trauma to them, and permitted as delicate cytologic definition as could be obtained by the supravital and section techniques. For these reasons, the differential counts of fixed smears were obviously less dependable than those by the other methods, and after preliminary trials, counts by that technique were excluded from the experimental correlations.

Methods. The guinea pig was selected as the experimental animal because of the similarity of its marrow to that of the human and the need of data concerning the normal proportions of its cells. Fifteen males and 4 females were used. They were kept in the animal room 3 to 25 days on a liberal diet consisting of oats, rabbit pellets (Ralston Purina Company) and daily rations of greens, with water available at all times. They weighed 290 to 500 gm. at the time of sacrifice and were all in excellent condition.

When ready for study, both thighs were shaved, and the pig was then anesthetized lightly with ether. Ten cc. of physiologic saline were immediately injected intraperitoneally, and 2 to 4 cc. of blood removed by cardiac puncture. During the next 10 to 15 minutes the anesthetic was maintained at the same level while the blood clotted and was centrifuged for the serum. A lethal dose of ether was then administered, and while it was taking effect, or immediately after death, one femur was removed. The bone was opened lengthwise by nipping with rongeurs with care not to compress the marrow. Blocks of marrow, about 5 x 3 x 3 mm., were scooped out with a scalpel and immediately dropped in the fixative recommended by Kingsley.[16] The other femur was then removed and opened in similar manner, and the marrow was aspirated into a 5 cc. syringe through a 20 gauge needle, both of which had been previously moistened with the guinea pig's serum. It was frequently necessary to separate the marrow into smaller pieces so that it would go through the needle. This was done by moving the needle back and forth within the marrow cavity or by gently fragmenting a specimen of marrow with the needle. A small amount of serum was drawn into the syringe after the marrow had been aspirated so that the final volume totaled 0.25 to 0.5 cc. The syringe was

* Now on active service with the Armed Forces.

then vigorously shaken. The resultant suspension of tiny fragments of marrow in serum was used for the supravital preparations, and for the marrow serum mixtures employed in the preparation of fixed smears.

The methods which were tried in the preparation of the fixed smears were as follows: (1) Marrow touched to cover-slips. (2) Scrapings of marrow spread on cover-slips with a scalpel. (3) Marrow-serum mixtures (described above) pulled between two cover-slips. (4) Cover-slips flooded with the marrow-serum mixtures and allowed to dry. (5) Cover-slips flooded with the marrow-serum mixtures, and immediately fixed with absolute methyl alcohol. (6) Cover-slips flooded with the marrow-serum mixtures, followed by withdrawal of as much of the serum as possible by suction, and drying at an angle of 45 degrees.

With the exception of Method 5, the smears made by each of the above methods were dried in air and then immediately fixed in absolute methyl alcohol, as recommended by Kingsley. They were stored in cardboard boxes and stained within 3 weeks of preparation. Both Kingsley's[11] and Wright's stains were used. The latter was employed in the standard strength and also after dilution to 50% with absolute methyl alcohol. The time for staining was varied with the Kingsley's stain, as recommended,[16] and under those conditions that stain yielded the better results. Differentiation of the staining, as described for sections, was unsuccessful.

The fixed blocks were dehydrated and embedded according to the technique recommended by Kingsley. The sections were cut routinely at 3 micra and stained according to the procedure outlined by Kingsley. The counts were made from consecutive fields.

The slides and cover-slips for the supravital smears were cleaned and stained as described by Cunningham and Tompkins.[1] The neutral red dye was used in the proportion of 0.9 cc. of a saturated solution in absolute alcohol to 10 cc. of absolute alcohol. A drop of the marrow-serum mixture on a cover-slip was inverted on a stained slide and rimmed with salvoline in the manner employed in making supravital blood smears. Upon macroscopic examination of the smears, there could be seen small, separate fragments of marrow which varied in size from 2 mm. in diameter to particles which were barely discernible. Under the microscope, the cells around the periphery of the smaller fragments were thinly spread and well stained. The differential counts were made in those areas, not too far peripherally where cells had wandered from the fragment, or been floated centrifugally, and not too far centrally where they were too tightly packed for accurate detail. The number of cells that could be counted on the periphery of a single fragment varied with the size and thinness of the fragment. Occasionally only one fragment was necessary to obtain the desired number of cells (see below), but usually at least two, and frequently more, were used. The spreads were protected from direct sunlight and frequently allowed to remain at room temperature for 30 to 45 minutes before use. The cells remained in a perfectly satisfactory condition during that period of time. This technique differs from that described by Sabin[20] in that intact fragments of marrow are examined, rather than freed cells. In many respects it is similar to the technique described by Isaacs[10] and differs mainly in the size of fragment employed for examination. Isaacs did not make counts of his supravital preparations but found that "the clearness of the picture obtained in this way is remarkable."

Binocular microscopes with apochromatic lenses were used for all counts. These were made under oil immersion with 2 mm. objectives and ×10 oculars, giving a magnification of ×900. In order to facilitate counting by division of the microscopic field into smaller units an appropriately marked glass disk was used in one ocular. A square was scratched in the center of the disk with a diamond pencil, and was divided into 4 smaller squares, the sides of which measured approximately 2.5 mm., and appeared as long with the magnification used as a distance of 0.04 mm. on the stage. Just about enough cells were circumscribed by each square to avoid the confusion met in trying to count a large crowded field. A mechanical stage was employed. Each observer

TABLE 1.—CHARACTERISTICS OF THE CELLS OF BONE MARROW

Cells	Shape	In sections (Kingsley's stain)					Stained supravitally (neutral red)					
		Cytoplasm		Nuclei			Cytoplasm			Nuclei		
		Color	Granules	Character	Shape	Nucleoli	Color	Granules	Vacuoles	Refractility	Shape	Nucleoli
Myeloblast	Round	Pale blue	0	Very vesicular	Round	Variable	Colorless	0	0	Unappreciable	Round	Variable
Myelocyte	Round	Paler blue	+ to ++++	Vesicular or reticular	Round or oval	Variable	Colorless	+ to ++++	0 to +	Unappreciable	Round or oval	Variable
Polymorphonuclear	Variable	Pale pink	++++	Reticular	Polymorphous	None	Colorless	++++	+ to ++	Slight	Polymorphous	None
Megaloblast	Round	Deep blue	0	Vesicular	Round	Usually 1	Pale straw	0	+	Unappreciable	Round	1
Erythroblast	Round	Slate or purple	0	Dense, cartwheel	Round	Chromatin clumps	Straw	0	+	Slight	Round	None
Normoblast	Round	Pink	0	Pycnotic	Varible	None	Yellow	0	0 to +	Considerable	Round	None
Lymphocyte	Round	Medium blue	0	Dense, clumped	Round	Variable	Colorless	0	+ to ++	Considerable	Round	Variable
Monocyte	...	...	...	...	...	...	Colorless	0	++++	Slight	Variable	None
Clasmatocyte	...	...	...	...	...	...	Colorless	Variable	++++	Slight	Variable	Variable
Reticular cell	Elongated	Colorless	0	Very vesicular	Elongated	Variable	Colorless	0	+	Unappreciable	Oval	Variable

0 = none; + = rare; ++ = moderate number; +++ = many; ++++ = very many.

TABLE 2.—DIFFERENTIAL COUNTS BY BOTH TECHNIQUES
(500 Cells Included in Each Count)

Cells	Observer	Number of experiment																	
		2		5		6		7		8		9		10		12		14	
		Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.
Myeloblasts	1	*4.6*	*10.8*	*2.1*	*5.2*	*7.2*		*3.3*		*5.0*		*4.4*		*2.9*		*3.4*	*2.1*	*1.3*	*3.*
	2	4.0		4.4		2.8		4.2		5.2		5.0		3.0		1.0	3.6	1.7	7.
Myelocytes	1	*29.0*	*14.6*	*27.1*	*9.6*	*15.4*		*8.1*		*10.0*		*29.2*		*16.1*		*7.3*	*10.0*	*8.2*	*9.*
	2	23.2		26.8		36.6		10.2		27.8		29.0		28.4		50.2	20.0	37.3	14.
P.M.N.	1	*16.0*	*30.5*	*35.8*	*40.1*	*37.6*		*28.3*		*29.9*		*15.8*		*26.3*		*15.6*	*21.0*	*13.0*	*23.*
	2	37.0		21.4		30.6		25.2		19.0		27.6		20.8		13.2	15.2	3.3	9.
Total myeloid cells	1	*49.6*	*55.9*	*65.0*	*54.9*	*60.2*		*39.7*		*44.9*		*49.4*		*45.3*		*26.3*	*33.1*	*22.5*	*37.*
	2	64.2		52.6		70.0		39.6		52.0		61.6		52.2		64.4	38.8	42.3	31.
Megaloblasts	1	*2.6*	*3.8*	*1.8*	*3.6*	*2.0*		*4.1*		*1.8*		*1.6*		*1.9*		*2.2*	*3.3*	*2.0*	*4.*
	2	2.0		2.4		2.0		3.2		4.8		3.6		2.2		1.2	5.2	3.1	3.
Erythroblasts	1	*6.8*	*9.0*	*6.0*	*15.0*	*19.1*		*31.0*		*21.2*		*22.4*		*21.2*		*11.3*	*15.6*	*7.2*	*15.*
	2	12.2		11.8		5.0		15.4		9.0		13.4		18.0		5.6	16.2	10.1	20.
Normoblasts	1	*30.6*	*19.4*	*15.1*	*16.4*	*8.3*		*15.9*		*23.7*		*15.6*		*23.1*		*44.2*	*32.5*	*44.7*	*42.*
	2	12.0		16.8		7.0		19.4		19.2		11.6		15.6		20.6	23.2	29.4	23.
Total nucleated erythroid cells	1	*40.0*	*32.2*	*22.9*	*35.0*	*29.4*		*51.0*		*46.7*		*39.6*		*46.2*		*57.7*	*51.4*	*53.9*	*58.*
	2	26.2		31.0		14.0		38.0		33.0		28.6		35.8		27.4	44.6	42.6	36.
Total myeloid and nucleated erythroid cells	1	*89.6*	*88.1*	*87.9*	*89.9*	*89.6*		*90.7*		*91.6*		*89.0*		*91.5*		*84.0*	*84.5*	*76.4*	*80.*
	2	90.4		83.6		84.0		77.6		85.0		90.2		88.0		91.8	83.4	84.9	87.
M/E ratio	1	*1.2*	*1.7*	*2.8*	*1.6*	*2.0*		*0.8*		*1.0*		*1.2*		*1.0*		*0.5*	*0.6*	*0.4*	*0.*
	2	2.5		1.7		5.0		1.0		1.6		2.2		1.5		2.3	0.9	1.0	1.
Lymphocytes	1	*6.4*	*10.0*	*6.3*	*4.2*	*6.8*		*6.5*		*5.6*		*6.6*		*5.6*		*8.3*	*12.7*	*5.1*	*9.*
	2	7.8		13.4		13.4		18.0		10.6		7.6		10.2		4.6	14.4	3.3	10.
Monocytes	1	*3.4*		*3.5*												*4.7*		*15.9*	
	2															0.6		9.6	
Clasmatocytes	1	*0.2*		*0.3*												*1.4*		*1.1*	
	2															0.8		0.6	
Megakaryocytes	1	*0.4*	*1.4*	*1.7*	*1.2*	*0.4*		*1.0*		*0.8*		*1.0*		*1.0*		*1.0*	*0.4*	*1.1*	*1.*
	2	1.8		1.2		1.6		1.2		1.2		1.4		0.2		2.2	0.4	1.5	1.
Reticular cells	1	*0.0*	*0.0*	*0.0*	*4.2*	*3.0*		*1.8*		*1.8*		*3.2*		*1.8*		*0.0*	*2.4*	*0.3*	*4.*
	2	0.0		1.8		1.0		3.2		3.2		0.8		1.6		0.0	1.6	0.1	1.

* Figures in italics, means obtained by Observer 1.

counted 500 cells in each preparation, both supravital and section, with the result that 1000 cells were counted by each observer, or that 1000 cells were counted by each method. Krumbhaar and Custer[17] have determined that this number of cells is usually sufficient for dependable counts of marrow. The counting was kept strictly individual without interchange of opinion concerning cellular identity or reference to other counts of the same marrow, in order to insure as severe a test of the individual techniques as possible.

TECHNIQUES AND BOTH OBSERVERS
1000–2000 in Each Experiment)

Number of experiment																					Mean by each observer		Mean of all counts	
15		16		17		18		19		20		21		22		23		24							
Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.	Sv.	Sec.			Sv.	Sec.	Sv.	Sec.
1.4	*4.0*	*4.0*	*4.2*	*4.1*	*3.6*	*3.4*	*2.2*	*3.2*	*1.0*	*2.0*	*2.1*	*2.6*	*3.1*	*1.8*	*2.6*	*1.8*	*3.0*	*1.6*	*2.0*			*2.7*	*3.9*	2.8	4.0
1.8	4.0	3.2	3.8	3.2	3.6	4.0	5.2	3.6	4.0	4.2	3.4	3.4	4.6	3.2	1.6	2.0	4.6	2.6	2.6			2.8	4.1		
11.0	*9.4*	*9.0*	*14.5*	*13.1*	*19.0*	*7.0*	*12.3*	*12.0*	*9.4*	*13.6*	*14.6*	*15.2*	*14.2*	*13.5*	*14.0*	*12.6*	*13.8*	*10.3*	*8.9*			*13.5*	*13.3*	19.0	18.2
17.0	16.4	31.2	17.0	24.0	27.4	17.6	30.8	18.4	23.8	28.4	18.6	19.6	39.6	16.2	18.0	12.0	12.6	21.0	19.4			24.4	23.1		
19.1	*32.3*	*17.9*	*35.4*	*18.2*	*28.8*	*20.1*	*37.7*	*15.5*	*41.3*	*27.4*	*34.1*	*28.7*	*37.0*	*31.1*	*35.2*	*29.2*	*40.8*	*24.0*	*19.5*			*22.3*	*31.3*	16.9	27.7
14.4	21.4	5.6	25.6	7.8	20.0	11.6	25.0	17.2	31.8	13.0	35.2	13.4	24.2	15.4	32.0	11.8	17.6	11.2	17.0			11.5	24.0		
31.5	*45.7*	*30.9*	*54.1*	*35.4*	*51.4*	*30.5*	*52.2*	*30.7*	*51.7*	*43.0*	*50.8*	*46.5*	*54.3*	*46.4*	*51.8*	*43.6*	*57.6*	*35.9*	*30.4*			*38.4*	*48.5*	38.6	49.9
33.2	41.8	40.0	46.4	35.0	51.0	33.2	61.0	39.2	59.6	45.6	57.2	36.4	68.4	34.8	51.6	25.8	34.8	34.8	39.0			38.7	51.2		
2.0	*2.0*	*2.2*	*2.2*	*2.0*	*1.0*	*1.8*	*1.6*	*2.2*	*2.4*	*1.8*	*1.2*	*1.7*	*2.1*	*2.2*	*1.4*	*1.2*	*1.0*	*0.6*	*2.5*			*1.9*	*2.3*	2.4	3.0
4.6	5.0	3.0	5.4	2.4	2.2	3.2	4.4	3.6	4.4	3.0	2.4	2.8	1.6	2.8	3.2	2.0	5.2	2.8	4.2			2.9	3.6		
6.8	*18.9*	*5.0*	*14.2*	*4.5*	*8.8*	*5.6*	*15.9*	*6.0*	*18.3*	*10.5*	*25.4*	*4.2*	*25.7*	*5.0*	*28.6*	*4.1*	*26.6*	*2.8*	*38.2*			*6.1*	*20.8*	6.5	16.7
9.2	9.4	6.8	8.6	7.0	8.8	7.0	7.0	4.8	7.2	4.4	5.2	6.6	6.6	6.2	8.8	7.8	21.4	5.8	25.0			6.8	12.5		
31.9	*21.5*	*32.3*	*20.1*	*26.2*	*25.2*	*26.0*	*25.0*	*29.9*	*18.7*	*25.6*	*12.7*	*21.6*	*9.4*	*17.7*	*9.2*	*27.4*	*3.4*	*19.0*	*15.1*			*28.0*	*18.0*	26.6	17.3
35.6	26.8	22.4	22.8	21.2	25.6	30.0	17.4	23.8	18.0	19.4	15.6	22.6	6.2	22.2	13.2	29.2	13.6	26.0	10.6			25.2	16.6		
40.7	*42.4*	*39.5*	*36.5*	*32.7*	*35.0*	*33.4*	*42.5*	*38.1*	*39.4*	*37.9*	*39.3*	*27.5*	*37.2*	*24.9*	*39.2*	*32.7*	*31.0*	*22.4*	*55.8*			*36.0*	*41.1*	35.5	37.0
49.4	41.2	32.2	36.8	30.6	36.6	40.2	28.8	32.2	29.6	26.8	23.2	32.0	14.4	31.2	25.2	39.0	40.2	34.6	39.8			34.9	32.8		
72.2	*88.1*	*70.4*	*90.6*	*68.1*	*86.4*	*63.9*	*94.7*	*68.8*	*91.1*	*80.9*	*90.1*	*74.0*	*91.5*	*71.3*	*91.0*	*76.3*	*88.6*	*58.3*	*86.2*			*74.4*	*89.6*	74.0	86.8
82.6	83.0	72.2	83.2	65.6	87.6	73.4	89.8	71.4	89.2	72.4	80.4	68.4	82.8	66.0	76.8	64.8	75.0	69.4	78.8			73.6	84.0		
0.8	*1.1*	*0.8*	*1.5*	*1.1*	*1.5*	*0.9*	*1.2*	*0.8*	*1.3*	*1.1*	*1.3*	*1.7*	*1.5*	*1.9*	*1.3*	*1.3*	*1.9*	*1.6*	*0.6*			*1.2*	*1.3*	1.2	1.6
0.7	1.0	1.2	1.3	1.1	1.4	0.8	2.1	1.2	2.0	1.7	2.5	1.1	4.7	1.1	2.0	0.7	0.9	1.0	1.0			1.2	1.9		
11.0	*10.0*	*15.3*	*7.0*	*8.8*	*8.8*	*12.3*	*2.6*	*12.4*	*5.9*	*10.1*	*6.2*	*7.2*	*5.6*	*14.5*	*5.2*	*9.5*	*6.8*	*22.4*	*9.9*			*10.7*	*6.9*	11.3	10.2
8.8	14.6	11.6	13.6	11.8	10.2	8.4	9.0	12.8	8.0	13.8	16.4	16.6	14.8	15.6	20.0	20.2	22.4	14.2	20.2			11.8	13.4		
9.6		*11.2*		*15.4*		*18.8*		*13.9*		*4.9*		*11.1*		*10.6*		*12.6*		*15.7*				*10.8*		11.0	
5.6		14.8		17.2		15.0		12.4		10.2		12.6		13.8		10.8		11.6				11.2			
2.0		*1.6*		*1.6*		*1.6*		*2.4*		*1.6*		*2.7*		*0.6*		*0.4*		*1.4*				*1.4*		1.4	
1.4		1.0		1.6		0.8		1.4		1.2		1.4		2.4		2.0		2.0				1.4			
2.8	*1.0*	*0.8*	*0.8*	*3.3*	*1.2*	*2.2*	*1.4*	*1.0*	*1.4*	*2.0*	*1.6*	*2.9*	*1.2*	*1.8*	*0.2*	*0.8*	*0.2*	*1.2*	*1.2*			*1.6*	*1.0*	1.6	1.1
1.6	1.6	0.4	1.8	2.6	1.2	1.4	0.8	1.2	1.2	1.8	1.4	0.8	1.0	1.2	0.8	1.4	0.6	2.2	0.2			1.5	1.1		
2.4	*1.9*	*0.4*	*1.4*	*2.2*	*3.8*	*1.2*	*1.0*	*0.8*	*1.4*	*0.6*	*2.2*	*1.8*	*1.5*	*1.0*	*3.2*	*0.4*	*4.2*	*0.4*	*2.5*			*0.8*	*2.4*	0.7	1.9
0.0	0.8	0.0	1.4	1.0	1.0	1.0	0.4	0.8	1.6	0.6	1.8	0.2	1.4	0.8	2.4	0.8	2.0	0.6	0.8			0.5	1.5		

The main morphologic criteria used in the identification of the cells by either technique are listed in Table 1. Differentiation of the younger cells in sections is inevitably dependent upon comparisons and evaluations relative to the staining of other cells in the same preparation, and there is no infallible way to differentiate individual early forms in stained preparations of bone marrow, if seen alone. This is achieved with considerable certainty, however, if the staining of any given section is considered as a whole.

It is recognized that the cytologic classification chosen is subject to controversy. It was chosen merely to facilitate comparison between the two techniques by synchronizing the nomenclature where possible, and no attempt is intended to uphold any specific concepts as to cellular origin. No attempt was made to separate either the myelocytes or polymorphonuclears into sub-groups.

Presentation and Discussion of the Experimental Data. Throughout the text the word "duplicate" is used to indicate two counts made by the same technique, but by different observers, on different specimens of the same marrow. The word "corresponding" is used to indicate two counts made by the supravital and section techniques, respectively, by the same observer on different specimens of the same marrow. The observers, and the counts by each, are referred to separately as the first and second, respectively.

The differential counts by each observer are listed in Table 2. Supravital counts were not made on animals 6 to 10 inclusive by Observer 1, and on animals 2 to 10 inclusive by Observer 2. Table 3[1] was compiled from Table 2 in order to facilitate evaluation of differences between counts. As defined in this table, a difference between counts is "statistically significant" if the "probability" of its occurrence by chance is less than once in 100 times.

The data may now be considered from the following aspects:

1. *The Variations Between "Duplicate" Counts by Different Observers Using a Single Technique (Tables 2 and 3).* This analysis is designed to determine the extent of personal differences in classification, irrespective of technique, and the degree to which susceptibility to such personal differences is influenced by technique.

TABLE 3.—MEAN "DUPLICATE" AND "CORRESPONDING" COUNTS, AND THE PROBABILITY* OF THE OCCURRENCE OF THE DIFFERENCES BETWEEN THESE MEANS†

	"Duplicate" counts (*i. e.*, same technique, 2 observers)						"Corresponding" counts (*i. e.*, 2 techniques, same observer)					
	Supravital			Sections			Observer No. 1			Observer No. 2		
	Mean			Mean			Mean			Mean		
Type of cell	Obs. 1	Obs. 2	Probability	Obs. 1	Obs. 2	Probability	Sv.	Sect.	Probability	Sv.	Sect.	Probability
Myeloblasts	2.7	2.8	0.81	3.9	4.1	0.87	2.7	3.9	0.06	2.8	4.1	<0.01
Myelocytes	13.5	24.4	<0.01	13.3	23.1	<0.01	13.5	13.3	0.92	24.4	23.1	0.72
Polymorphonuclears	22.3	11.5	<0.01	31.3	24.0	<0.01	22.3	31.3	<0.01	11.5	24.0	<0.01
Sum	38.4	38.7	0.94	48.5	51.2	0.40	38.4	48.5	<0.01	38.7	51.2	<0.01
Megaloblasts	1.9	2.9	<0.01	2.3	3.6	<0.01	1.9	2.3	0.17	2.9	3.6	0.11
Erythroblasts	6.1	6.8	0.40	20.8	12.5	<0.01	6.1	20.8	<0.01	6.8	12.5	<0.01
Normoblasts	28.0	25.2	0.33	18.0	16.6	0.51	28.0	18.0	<0.01	25.2	16.6	<0.01
Sum	36.0	34.9	0.75	41.1	32.8	<0.01	36.0	41.1	0.10	34.9	32.8	0.54
Lymphocytes	10.7	11.8	0.56	6.9	13.4	<0.01	10.7	6.9	<0.01	11.8	13.4	0.35
Monocytes	10.8	11.2	0.84	....	....	....	10.8	....	....	11.2		....
Clasmatocytes	1.4	1.4	0.92	....	....	....	1.4	....	....	1.4		....
Megakaryocytes	1.6	1.5	0.72	1.0	1.1	0.31	1.6	1.0	<0.01	1.5	1.1	0.05
Reticular cells	0.8	0.5	0.21	2.4	1.5	<0.01	0.8	2.4	<0.01	0.5	1.5	<0.01

* This probability is a measure of how often the observed differences might be reached or exceeded by chance. When the probability is less than 0.01 (*i. e.*, one time in 100), the difference is regarded as "statistically significant" (*i. e.*, not likely to occur as a matter of chance). Values obtained from Fisher's[7] table of values of t.

† These mean values are obtained from Table 2.

It is obvious from Table 3 that there is less variation between the duplicate supravital counts than between the duplicate section counts. In fact, with the exception of the myelocytes, polymorphonuclears and megaloblasts, the differences between the means of the duplicate supravital counts are not "statistically significant," while those between the means of the duplicate section counts are "significant" in most instances. Further analysis of the table shows that while the deviations between the means of the duplicate counts of myelocytes and polymorphonuclears are "significant," by either technique, the deviation between the means of the duplicate counts of total myeloid cells is "insignificant" in the supravital counts, and relatively "insignificant" in the fixed counts. The variations between the duplicate counts of myelocytes and polymorphonuclears must, therefore, be dependent largely upon "personal factors" (chiefly upon lack of agreement on the part of the two counters in differentiation between the members of the myeloid series) and not upon the limitations of the methods employed. This is borne out by the fact that the differences between the duplicate counts of these cells occur in a single direction in practically all counts (Table 2).

The same conclusion applies to the differences between the duplicate counts of megaloblasts by either method, since they also occur almost uniformly in one direction (Table 2).

Analysis of the differences between the duplicate section counts of the remaining cells of the marrow indicates that they also are dependent largely upon personal differences in cytologic differentiation. The differences between the duplicate section counts of erythroblasts are almost consistently in one direction (Table 2), but, unlike the myeloid series, they are not counterbalanced by the other members of the erythroid series and therefore the means of the total erythroid counts also differ "significantly" (Table 3). The differences between the duplicate section counts of erythroblasts occur almost uniformly in the opposite direction to the differences between the duplicate counts of lymphocytes and are of the same order of magnitude (Table 2). It seems probable, therefore, that lack of agreement on the part of the two counters in differentiation between these two types of cells in sections represents the "personal factor" responsible for the variations in the duplicate section counts of not only erythroblasts and lymphocytes, but of all the other values dependent upon them (total nucleated red cells, combined myeloid and erythroid cells, and M/E ratios).

This analysis, therefore, indicates that when "statistically significant" differences occur by either technique between duplicate counts by different individuals they are the result of differences in cytologic differentiations by the individuals, and are therefore controllable. Such "personal differences" apparently occur more easily with the section technique.

2. *Comparison Between "Corresponding" Supravital and Section Counts by a Single Observer* (*Tables 2 and 3*). This analysis is designed to bring out points of difference in counts made by the same individual

by the two methods, the reasons for difference, and the relative dependability of counts by each technique.

From Table 3 it is seen that, with the exception of the lymphocytes, the differences which occur between corresponding counts by the two methods are in the same direction for each observer and may, therefore, be regarded as due to differences of technique and not to "personal factors." These differences are discussed in groupings based on probable interrelationships.

The percentages of both myeloblasts and, to less extent, megaloblasts tend to be slightly greater in the section counts (Table 2). The percentage of erythroblasts is greater, and that of normoblasts less in the section counts, although the total percentage of nucleated red cells is usually the same by either method. These three facts, as well as the irregularities in the "duplicate" lymphocytic counts, all seem to be explainable on the same basis. In supravital preparations, differentiation of the erythroid series depends upon the quantity of hemoglobin detectable in the unstained living cell; in sections, differentiation of cellular types depends upon the character of the nuclei and the degree of basophilia of the cytoplasm, coupled with the presence or absence of cytoplasmic inclusions. The delicacy of detection of these factors in sections is dependent upon the extent of crowding of the cells and upon the depth and differentiation of staining. The less complete the differentiation, the more pronounced becomes the basophilic side of the picture, *i. e.*, density of nucleus and blueness of cytoplasm, and the less the acidophilic side, *i. e.*, granules and hemoglobin. Consequently there is a tendency toward overshadowing of the earliest acidophilic characteristics in fixed staining with the result of an apparently slightly increased proportion of the youngest cells in section counts. For the same reason there is an apparent shift between erythroblasts and normoblasts in favor of the former. Finally, the shift toward basophilia causes difficulty in the differentiation between lymphocytes and erythroblasts. It is probable that this is the factor involved in the lack of agreement between the "duplicate" counts of these cells which was discussed previously. The fact that the difference between the means of the "corresponding" counts of lymphocytes is reversed for the two observers (Table 3) may reflect greater difficulty for one of them in differentiation between these cells in sections.

The percentages of polymorphonuclears are consistently higher in the section counts, while the percentages of myelocytes are relatively the same in both counts. Consequently, the percentages of total granulocytes are proportionately higher in the section counts, and therefore also the combined total percentages of erythroid and myeloid cells, and the M/E ratios.

There are two factors which should be considered as possible explanations of this difference in polymorphonuclear counts by the two methods. First, the manner of making the supravital counts was such that the more motile and more easily disturbed polymorphonuclears might have been floated peripherally from the fragments of marrow upon which the counts were made, and thus have been excluded from

the counts. Since the divergence of the supravital counts from the fixed counts is so constant, this explanation seems unlikely, as floating of the same proportion of cells in every smear would hardly be expected. Second, the average increase in the polymorphonuclears in the section counts (10.8%) is practically equal to the mean percentage of monocytes in the supravital counts (11%). Monocytes are recorded only in the latter counts. The nuclei of monocytes are frequently similar to those of the younger polymorphonuclears. Both cells have cytoplasmic granules in fixed material, and the cytoplasm itself is frequently indiscernible in crowded areas. For these reasons it is probable that inclusion of monocytes with polymorphonuclears accounts for the consistently greater proportion of polymorphonuclears by the section technique.

The percentage of reticular cells is greater in the section counts (Table 2), the average increase (1.3%) being equal to the percentage of clasmatocytes in the supravital counts (1.4%). As in the case of monocytes, clasmatocytes are recorded only in the latter counts. Reticular cells and clasmatocytes generally assume a similar elongated shape. Their nuclei are fairly similar and their cytoplasm equally indefinable unless characterized by inclusions. From these various considerations, therefore, it seems probable that the increase in reticular cells in the fixed counts is due to inclusion of clasmatocytes with counts of reticular cells.

3. *The Marrow of the Normal Guinea Pig* (based on the average of the combined counts by both observers. Last column, Table 2). Seventy-five per cent of the cells in supravital counts of the marrow of normal guinea pigs, and 85% in fixed counts, are comprised of the combined myeloid and erythroid cells. Lymphocytes comprise roughly 10% by either method, and the sum of the reticular cells, clasmatocytes and giant cells, 5%. Finally, monocytes comprise 10% in supravital counts while their inclusion with polymorphonuclears in section counts, as has been discussed, is probably responsible for the higher value of the sum of the myeloid and erythroid cells in the section counts.

The percentages of both myeloblasts and megaloblasts are slightly greater in section than in supravital counts. By either method alone, however, they equal each other and are less than the percentage of any other cells of their respective series.

There was considerable discrepancy on the part of the two counters in differentiation between myelocytes and polymorphonuclears, respectively, as has been discussed under heading "1." Therefore, although the average combined percentage of myelocytes by either method is the same, the discrepancy in classification makes it impossible to formulate any statement concerning the normal proportion of myelocytes to polymorphonuclear cells.

The ratio of normoblasts to erythroblasts varies with the technique, although the total number of cells of the erythroid series is uninvolved. In the supravital counts there are four times as many normoblasts as erythroblasts. In the section counts, the per cent of normoblasts is less than that of erythroblasts. That the anesthesia may have influ-

enced the erythroid values is possible, but not very probable, in view of the short period in which it was in effect before the specimens were obtained.

While slightly higher by the section than by the supravital technique, the M/E ratios fluctuate around unity. This figure is low compared to the ratios obtained by other workers with other species, as reviewed by Sabin.[22] The fact that the M/E ratio represents the proportion of all myeloid cells (mature polymorphonuclears ready for emigration as well as immature myelocytes) to only the immature nucleated red cells, irrespective of the number of possibly newly formed erythrocytes, seems to throw question upon the wisdom of too rigid an interpretation or comparison of this value.

Discussion. Isaac's[9] observation that the cells of marrow stain more intensely and more like blood cells when exposed to serum is significant in evaluation of techniques for the study of marrow. He found[10] that the morphology of cells prepared by the touch technique differs from that of blood cells evidently for lack of suitable moistening. With this exception, mixture with more or less serum obviously occurs with most of the techniques used for the preparation of smears of marrow.

Custer,[2] Tuohy and Gillespie,[23] Dameshek,[3] Jones,[11] Kato,[13] Krumbhaar and Custer,[17] and Gall,[8] as well as others, obtained satisfactory cytologic detail with fixed smears prepared by either smearing marrow on slides or by the touch technique. The latter 3, however, believe that differential counts made by these techniques are inaccurate, and that, as Peabody[19] first suggested, study of sections is more dependable for estimations of relative cellularity. Morris and Falconer,[18] on the other hand, compared smear preparations of aspirated marrow to sections and state that "smears and sections checked well." They are not specific, however, as to whether reference is had to qualitative or quantitative comparisons.

Sabin, Miller, Smithburn and Hummel[21] made duplicate counts of rabbits' marrow from fixed and supravital films and obtained good correlation in half of the instances. In their opinion the supravital technique is the better standard. Doan and Zerfas[5] used both methods with satisfaction. Gall[8] found the supravital, as well as the touch technique, unsatisfactory for counts. Jones[12] agrees in regard to the former technique, but not in regard to the latter.

Custer,[2] as well as Dameshek, Henstell and Valentine[4] and Doan,[6] suggest that adequate study of the marrow should include more than one technique. Krumbhaar and Custer[17] suggest, further, that comparative counts should be made, not only by the same techniques, but by the same person. The concluding words of Doan and Zerfas[5] in their enumeration of the factors which give rise to this variety of opinions concerning techniques are illustrative of the status up to the present time: ". . . . all of these, and more, make it necessary to be very conservative in the drawing of deductions from any one limited series of observations or in trying to compare the figures obtained from different investigators."

In the studies reported here, a variable degree of trauma and inade-

quate distribution were found to be inevitable in fixed smears of marrow, and consequently to invalidate differential counts made from them. Detailed comparison between differential counts by different methods was therefore carried out only on counts from sections, and from small pieces of marrow stained supravitally. From the consideration of the data it seems apparent that fairly dependable differential counts of marrow are possible by either of these techniques. There are certain differences in counts by the two methods, however, which are inherent in the techniques and which must be recognized and given due evaluation.

The supravital technique permits of closer agreement between duplicate counts and of surer differentiation of all types of cells. It permits the detection of the two phagocytic mononuclear cells, clasmatocytes and monocytes, and it facilitates the study of certain of the physiologic activities of marrow cells. On the other hand, it permits study of only the hematopoietically active areas of the marrow, and gives little information concerning the frequency of such areas.

The section technique permits of sufficiently close agreement between duplicate differential counts for most purposes, and offers the advantage of a general survey of the marrow as a whole, hematopoietic centers as well as inactive ones. It does not permit of differentiation of the phagocytic mononuclear cells, which obviously results in a corresponding inaccuracy elsewhere (probably in the counts of polymorphonuclear and reticular cells).

There are various technical factors in the preparation of sections for counting which require great care if the maximum accuracy is to be obtained. If these are neglected the sections may actually mislead, since errors in technique may cause incorrect classification of cells. Meticulous adherence to the proportions stipulated by Kingsley[16] for the fixative has been found to be perhaps the most important factor involved. A mistake at that stage is irrevocable and can lead to subtle errors in classification that are extremely difficult to detect. Most important among these is a granular precipitation of hemoglobin which results in confusion between nucleated red cells and granulocytes.

Inadequate staining may also lead to errors in differentiation, but they are rectifiable by use of new sections. Experience with the stain and the degree of differentiation necessary for accurate differential counting are all that are needed to avoid such errors and they will therefore not be discussed in minutiæ.

We agree with those who advocate that examination of marrow should include the use of more than one technique. However, where that is impracticable, relatively dependable differential counts may be made by either of the techniques employed in these studies; but those by the supravital technique are subject to less error, and offer more information. Conclusions based upon the counts made with either technique must be made with cognizance of the sources of error in each, and especially with full appreciation of the fact that a differential count of small portions of marrow represents the relationship between cells in active hematopoietic areas, and does not necessarily offer information as to the relative frequency of such areas.

Summary. 1. A method is described for counting the hematopoietic cells of small pieces of bone marrow supravitally.

2. Counts of bone marrow of normal guinea pigs have been made from (1) fixed smears prepared by a variety of methods; (2) small pieces of marrow stained supravitally; and (3) sections fixed and stained by Kingsley's method.

3. Inadequate distribution and trauma are found to be inherent sources of error in the use of fixed smears. Differential counts made from them are therefore less dependable than by the other two methods. The data by the other two methods have been analyzed statistically.

4. The difference between section counts of the same marrow by different observers is greater than between supravital counts under the same conditions. Both methods, however, are sufficiently reliable for studies based upon comparative, rather than absolute, values.

5. There are certain differences between counts of the same marrow by the section and supravital techniques, respectively, which must be taken into consideration in evaluation of any count of bone marrow. These are inherent in the techniques and have been analyzed for the probable explanation. The most important differences between section and supravital counts are: (1) a greater percentage of polymorphonuclear cells in section counts; (2) inability to differentiate monocytes and clasmatocytes, specifically, in section counts; and (3) a shift toward the younger cells of any series in section counts. The method used in any study, therefore, should always be stated.

6. The mean differential count of the bone marrow of normal guinea pigs is established, and certain generalizations concerning the proportional relationships are formulated from it.

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TOXIC EFFECTS OF SULFONAMIDES ON THE EYES

By H. P. Wagener, M.D.

With the main purpose of estimating the probable efficacy of various sulfonamides in the treatment of extra- and intraocular infections, a number of investigators have made determinations of the penetration of these drugs into the tissues of the eye. The relative concentration in the different tissues and fluids may have some bearing on the toxic as well as on the curative effects.

Bellows and Chinn[4,5] found that, in dogs, after a single massive dose of sulfanilamide by mouth (0.2 gm. per kilo of body weight), traces of the drug could be detected in the eye within 15 minutes. Maximum concentration was reached within 6 hours in all the tissues examined except the lens in which maximum concentration occurred in 12 hours. The concentration of sulfanilamide was found to be in the chorioretinal tissue 80%, in the corneo-scleral tissue 65%, in the aqueous 60%, in the lens 50%, and in the vitreous 45% of the concentration in the blood which reached 19.1 mg. per 100 cc. Elimination of the drug from the lens and vitreous was relatively slow so that at the end of 24 hours, the concentration in these tissues was about 120% of that in the blood. When divided therapeutic doses were given, the relative concentrations were essentially the same as after a single massive dose in the aqueous, vitreous and chorioretinal tissues but were definitely higher in the lens and corneo-sclera, 100% and 150% of the blood levels respectively. The concentration in the aqueous was not increased by the local application of heat, dionine, atropine or eserene but was increased by the instillation of mecholyl and by paracentesis, both of which increased the amount of protein in the aqueous probably by increasing the permeability of the capillaries in the ciliary body. In 2 patients who were taking 3 gm. of sulfanilamide by mouth daily 0.8 mg. per 100 cc. and 1.5 mg. per 100 cc. were found in the tears. Bellows and Chinn[4,5] were of the opinion that the drug passes into the tissues by simple diffusion and the tissues do not act to concentrate the drug. The sulfanilamide passes into the aqueous and vitreous by dialysis from the blood and

enters the lens from the surrounding media. Mengel[20] found a concencentration of 1.5 to 3.2 mg. per 100 cc. in the aqueous and vitreous of 2 blind human eyes after the administration of sulfanilamide orally.

Meyer, Block and Chamberlain[21] found that, after the administration of 1.2 gm. of sulfapyridine by mouth to rabbits, the concentration in $4\frac{1}{2}$ hours was in the blood 20 mg. per 100 cc., in the aqueous 14 mg. per 100 cc., and in the cornea 8.5 mg. per 100 cc. After $7\frac{1}{2}$ hours the concentration was in the blood 10 mg. per 100 cc., in the aqueous 7.6 mg. per 100 cc., and in the cornea 15.7 mg. per 100 cc. Bellows and Chinn[4,5] found that 4 hours after the administration to dogs of 0.2 gm. of sulfapyridine per kilo of body weight the blood concentration was 6.1 mg. per 100 cc. The concentration in the sclera was 114%, in the cornea 110%, in the chorioretina 100%, in the vitreous 96%, in the aqueous 80%, and in the lens 18% of the blood concentration.

Bellows and Chinn[4,5] made determinations also of the amount of sulfathiazole in the tissues of the eye in dogs 4 hours after the administration of 0.2 gm. per kilo of body weight. They found that the concentration in the blood was 7.4 mg. per 100 cc. and that the concentration in the sclera was 140%, in the chorioretina 66%, in the cornea 27%, in the vitreous 13%, in the aqueous 5.2%, and in the lens 0% of that in the blood.

Scheie and Souders[27] made a comparison of the concentration of sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine in the aqueous after a single massive dose orally. In 6 hours the aqueous contained 72% of the blood level of sulfanilamide, 62.8% of sulfapyridine, 48% of sulfadiazine, and 17% of sulfathiazole. In 24 hours, the aqueous contained 78% of the blood level of sulfanilamide, 81% of sulfapyridine, 75% of sulfadiazine, and 18% of sulfathiazole. The secondary aqueous after paracentesis contained 80% of the blood level of sulfanilamide, 88% of sulfapyridine, 80% of sulfadiazine, and 59% of sulfathiazole. Scheie and Leopold[28] found that in eyes with experimentally produced corneal ulcers the concentration of sulfathiazole in the aqueous rose to 46% of that in the blood and in eyes with experimental infections in the vitreous to from 82% to 91% of the blood level. Also the local instillation into the eyes of mecholyl or histamine caused a rise in the aqueous to from 30% to 50% of the blood concentration. Liebman and Newman[18] obtained comparable results from a similar investigation. In the primary aqueous the concentration of sulfanilamide was 67% of that in the blood, of sulfapyridine 64%, of sulfadiazine 55%, and of sulfathiazole 17%. In the secondary aqueous the concentration of sulfanilamide in the aqueous was 96% of that in the blood, of sulfapyridine 98%, of sulfadiazine 102%, and of sulfathiazole 41%. Liebman and Newman[18] stated that analysis of the eyes obtained at necropsy from 2 patients who had received sulfapyridine and 2 who had received sulfathiazole yielded results similar to those obtained in rabbits' eyes.

Alvaro[1] stated: "The fact that sulfonamide compounds penetrate into the tissues of the eye in concentrations only somewhat lower than the concentration in the blood, and the demonstration of the potential absorption by the several parts of the eyeball, make it easy to understand why there is a comparatively large group of ocular changes which have been attributed to these drugs." Among the toxic manifestations which are reported to have occurred in the eyes, Alvaro[1] mentioned edema of the lids and conjunctivæ, conjunctivitis, scleral reactions, cells in the aqueous, iritis, cataract formation, mydriasis, hemorrhagic extravasations in the retina, edema of the retina, optic neuritis, reduction in the fields of vision,

blurred vision of undetermined etiology, and transient changes in refraction. To these may be added the partial paralysis of the oculomotor nerve which was reported by Pitter[23] to have occurred during sulfanilamide therapy of gonorrhea. It is of interest to note, however, that with the exception of transient changes in refraction, reports of toxic manifestations in the eyes have been very infrequent. It seems probable that the relative infrequency of reports of the occurrence of conjunctivitis is due to the minor nature of the lesion and to its usual association with toxic lesions of the skin.

All of the changes in refraction reported to date have been in the direction of myopia except in the case described by Thorne[31] in which vision was improved from 20/200 to 20/20 in each eye by +3.50S and +.50Cyl ax 180. Since Thorne[31] suggested that the change in refraction might be due to spasm of the ciliary muscle, one wonders whether the minus sign may not have been inadvertently changed to plus in transcription. So far there has not been a very satisfactory explanation of the mode of development of these transient changes in refraction. The theory of spasm of the accommodation seems to have been pretty well disproved. The explanation of the myopia on the basis of edema of the lens, probably allergic or angioneurotic in origin, has been advocated by Spellberg,[30] Mattsson,[19] Gailey,[13] Bristow,[9] Rittenhouse,[25] Spearman,[29] Hornbogen,[17] and Blankstein.[6] Rittenhouse[25] has suggested the possibility that rapid absorption of the drug may bring about a change in water and salt metabolism which would affect the lens. He suggested also a possible toxic irritation of the parasympathetic fibers of the ciliary muscle. According to Rittenhouse,[25] Nitti and Bovet and Hageman and Blake were not able to produce sensitivity to sulfanilamide under experimental conditions. Blankstein[6] suggested that the edema of the lens might be the result of an osmotic change produced by the unequal distribution of the drug in the various tissues of the eye. Krause suggested, according to Blankstein,[6] that a low hydrogen ion content of the blood, which occurs at times during sulfonamide therapy, might activate the osmotic power of the lens tissue and favor its swelling.

Alvaro[1] collected 36 cases of transient myopia. Of these, 33 occurred during the course of sulfanilamide therapy and 3 during treatment with sulfapyridine. The amount of myopia developed in the sulfanilamide cases varied from 1.50 D to 4.50 D and averaged 2.60 D. In the 3 sulfapyridine cases, it was 2.00 D, 6.50 D, and 7.00 D, averaging 5.25 D. The amount was often different in the two eyes but both eyes were affected always. Men were affected slightly more often than women. The age incidence was as follows: below 20, 9.5%; between 20 and 30, 38.1%; between 30 and 40, 23.8%; and over 40, 28.6%. The smallest amount of sulfanilamide which had been taken before the onset of myopia was 0.3 gm. and the largest 42 gm. The smallest amount of sulfapyridine was 5 gm., and the largest 85 gm.; 36.36% of the individuals had taken less than 2 gm. of the drug; 13.6% between 2 and 4 gm.; 13.7% between 4 and 10 gm.; 22.6% between 10 and 20 gm.; and 13.7% more than 20 gm. The time of onset of the myopia after starting sulfanilamide varied from 12 hours to 14 days and in the sulfapyridine from 14 to 30 days; 36.36% of the cases occurred within 24 hours after the drug was started; 13.6% between the 1st and 4th days; 4.5% between the 4th and 9th days; 18.1% between the 10th and 20th days; and 22.7% after the 20th day. In 8 cases the myopia occurred after the drug had been stopped and started again in a second course. In only 1 of these cases had the drug been stopped because of the appearance of general signs of intolerance. There seemed to be

some relationship between the amount of myopia developed and the amount of the drug taken, the duration of treatment, and the age of the patient. Patients who had taken up to 2 gm. of the drug developed an average of 2.00 D of myopia; between 2 and 10 gm. 3.37 D, and over 10 gm. 4.17 D. Patients who had taken the drug for 24 hours or less developed an average of 2.00 D of myopia; between 2 and 5 days 3.16 D, between 6 and 15 days 4.60 D, and over 15 days 6.50 D. Patients below the age of 20 developed an average of 5.00 D of myopia; between the ages of 20 and 30, 4.29 D; between 30 and 40, 2.58 D; and over 40, 2.40 D. The myopia lasted for from 1 to 15 days (averaging 4.9 days). No relationship could be established between the amount of the drug taken and the duration of the myopia. All of the patients recovered without sequelæ.

With the exception of the cases reported by Hornbogen[17] and by Esterman[11] in which edema of the lids and conjunctivæ accompanied transitory myopia occurring in the course of sulfanilamide therapy, all the toxic conjunctival and scleral reactions have been described in cases under treatment with sulfathiazole.* Haviland and Long[16] reported that in 6 of 78 cases receiving sulfathiazole, there occurred between the 4th and 16th day of treatment conjunctival and scleral injection, restricted in the main to the exposed portions of the bulbar conjunctiva and sclera with burning and watery discharge. In 4 of the 6 cases the eye lesions occurred in association with skin lesions. In 3 cases the eye nearest the light was involved solely or primarily. The palpebral conjunctiva was involved also in the 2 cases most exposed to light. However, there was no "after sensitivity" to light and no recrudescence on exposure to light after discontinuance of the sulfathiazole. Exposure to light did not invariably bring on the conjunctivitis. No definite relationship could be established between the onset of the conjunctival reaction and the blood level of the drug. In 1 patient the conjunctival reaction recurred on readministration of the drug. Volini, Levitt, and O'Neil[34] reported that conjunctival reactions occurred in 5 of 180 patients receiving sulfathiazole, in 4 in association with skin reactions. The average dose taken by the affected patients was 34 gm., and the concentration of sulfathiazole in the blood averaged 4.2 mg. per 100 cc., varying from 2.5 to 7.1 mg. per 100 cc. The conjunctivitis was severe frequently with bright red injection of the palpebral and bulbar conjunctiva, edema of the eyelids, photophobia and seropurulent discharge. It cleared up rapidly without residuals after the drug was stopped. Volini[34] thought that immediate discontinuance was indicated on the appearance of the conjunctival reaction. Turkell and Wilhelm[32] observed 7 cases of conjunctivitis during administration of sulfathiazole. Quantitative determination of the sulfathiazole concentration in the tears was made in 4 of these 7 and in 10 other patients receiving sulfathiazole but without conjunctivitis. Sulfathiazole was found in the tears of all 14 in concentrations varying from 0.1 to 0.98 mg. per 100 cc. But the amount in the tears was not proportional to the blood concentration but was influenced by the secretory function of the lacrimal gland. In all cases of conjunctivitis, the sulfathiazole concentration in the tears reached 0.6 mg. per 100 cc. or more. However, in 6 of 10 cases without conjunctivitis, the sulfathiazole concentration was 0.56 mg. per 100 cc. or more. The conjunctivitis was not caused apparently by an increased concentration of sulfathiazole in the tears. According to Turkell and Wilhelm,[32] "The conjunctivitis occurring during the administration of sulfathiazole is, in all likelihood, a reaction peculiar to the drug. Although sulfanilamide has been found in the tears in greater concentration than

* See postscript following references p. 268.

sulfathiazole, conjunctivitis has not been observed." Nor has it been reported during the administration of sulfapyridine. Four of the 7 patients had no conjunctivitis during the first course of therapy. The fact that conjunctivitis occurred during the second or a subsequent course of treatment suggested sensitization to the drug. The conjunctivitis cleared up in 1 to 4 days after discontinuance of the drug without serious sequelæ. Turkell and Wilhelm[32] regarded the development of conjunctivitis as an indication for stopping the drug. However, in 1 case, the drug was not stopped and the conjunctivitis cleared up in 4 days under treatment with zinc sulfate. The author has observed several cases similar to those described by Haviland and Long.[16]

Morrison[22] reported the development of an allergic edema and inflammation of the lids and conjunctiva which developed during the treatment of a chronic catarrhal conjunctivitis with a 5% sodium sulfathiazole ointment in lanolin petrolatum. The eyes were well 5 days after the drug was stopped. The edema recurred in one eye when the drug was reapplied to that eye only while the ointment base alone was applied to the other eye.

Four cases of acute optic neuritis have been reported in patients under treatment with sulfonamide compounds. Bucy[10] described reduction of vision to 6/200 with the right eye and 4/200 with the left eye with relative central scotomas and some blurring of the disk margins in a 16 year old girl who had received 14.4 gm. of sulfanilamide for chronic recurrent osteomyelitis with some other evidences of intolerance to the drug. The vision cleared up in 2 days after stopping the drug. Alvaro[1] records also (1) a case described by Valdeavellano,[33] a girl aged 18, who showed blurring of the vision with slight changes in the optic disks after receiving sulfanilamide orally and parenterally for 12 days and who recovered normal vision in a few days after stopping the drug; (2) a case described by Guerra,[15] a man who had received 17 gm. of some drug of the sulfanilamide group for gonorrhea. He was completely blind with dilated pupils, blurred optic disks and dilated congested retinal veins. Twenty-seven days after the drug was discontinued, vision was 20/30 in each eye; but the fields of vision were still contracted and the light sense and color sense were below normal; (3) a case observed by Bresser,[8] a man, aged 19, who had received 8 gm. of sulfapyridine in a period of 3 days. He had blurred vision without refractive error and with ophthalmoscopic evidences of optic neuritis. The vision was 20/20 in each eye 17 days after the drug was discontinued. A case which belongs probably in the same general category was reported by Bloom, Leech and Shaw.[7] A man, aged 26, had received 22 gm. of sulfathiazole in 84 hours. His vision was reduced suddenly to the ability to see hand movements at 6 inches. Ophthalmoscopic examination revealed tortuosity and dilatation of the retinal vessels especially the veins, small petechial hemorrhages, and multiple small white crystal-like spots throughout the retina but concentrated especially around the macula. The sulfathiazole concentration in the blood was 1 mg. per 100 cc. The patient was placed on a milk diet and was given intravenous glucose in distilled water, citrocarbonate, vitamin B complex, vitamin K, and calcium gluconate. The vision returned to normal in 12 days. It seems possible that this may have been some form of angiospastic retinitis since the blood pressure rose with the onset of the loss of vision to from 174/90 to 178/120. One week later it was 128/88 and in another week was 110/68. The patient was under treatment for cystitis, pyelitis, and renal colic.

Only two reports have appeared in the literature of hemorrhagic extravasations into the retina in the course of sulfonamide therapy. The author

has observed several cases of this type in patients at the Mayo Clinic. Unfortunately no record was kept of the particular drug used in the individual cases. The hemorrhagic areas were of the thick so-called "purpuric" type. Baker[3] reported the case of a man, aged 36, who received 370 gr. of sulfanilamide in 4 days for the treatment of pyelitis with ureteral calculi. Vision was reduced to 20/60 in each eye. Ophthalmoscopic examination revealed "ball-shaped tufty hemorrhages along the smaller arterioles." One was at the center of each macula. The hemorrhages cleared up in 1 month. Blood studies were essentially normal, and Baker considered that the hemorrhages were due to increased permeability of the vessels of the retina. Goar[14] reported hemorrhagic extravasations into the retina in 2 patients both of whom were under treatment with sulfathiazole. A woman, aged 34, had received 78 gm. of sulfathiazole in 8 days for the treatment of a pneumonia due to the Type 1 pneumococcus. In the right retina there were 4 globular hemorrhages close to the optic disk; in the upper temporal quadrant of the left retina was a preretinal hemorrhage 3 disk diameters in size. One week later the vision was worse; there was a discrete spherical hemorrhage in each macula. At the end of a month, vision was 20/30 with the right eye and 20/100 with the left. The blood had been absorbed to a great extent. There was no evidence of blood dyscrasia or of renal or vascular disease. In Goar's second case, a woman, aged 37, had received 10.5 gm. of sulfathiazole in 3 days when she complained of blurred vision. Three weeks later, vision was 20/400 with the right eye and 20/25 with the left. Ophthalmoscopic examination revealed in the right macula an area of destruction of the retinal elements and below this a cherry red spot of hemorrhage. The left retina was normal. One week later, vision in the eye was 20/30. Most of the blood had been absorbed from the retina. There was some pigment proliferation in a discrete circular area in the macula.

Three studies have been made which might be said to estimate the visual efficiency of individuals receiving sulfonamides without any gross evidences of toxic reactions. Rosenthal[26] gave 90 to 195 gr. of sulfanilamide to 2 experimental subjects and found that the angioscotomas in the campimetric fields were reduced in width and length. They returned to normal in 4 or 5 days. This change in the scotomas was not due to oxygen deprivation since oxygen deficiency tends to widen the scotomas and since the angioscotomas in the individuals receiving sulfanilamide were not increased in size by giving oxygen. Rosenthal[26] postulated a relative increase in the oxygen present in the region of the retinal synapse or a possible specific influence of the sulfanilamide directly or indirectly on the synapse and on the retinal nutritive state. Alvaro[1] and Silva examined 14 patients for visual acuity, light and color sense, accommodation, muscle balance, state of the media and fundi, ocular tension, and pressure in the central artery of the retina before and after treatment with sulfanilamide, sulfapyridine, and sulfathiazole. No changes were noted in the visual acuity, light and color sense, state of the media and fundi, ocular tension, or pressure in the central artery of the retina. In 1 case, after 30 gm. of sulfathiazole, an exophoria of 4° was reduced to 2°, and in 1 case under sulfapyridine therapy an esophoria of 2° was reduced to 1°. In the other patients muscle balance was not affected. In all but 1 case, however, the power of accommodation was reduced. In 3 cases under sulfacetamide there was an average reduction in accommodative power of 1.33 D. In 4 cases under sulfanilamide with an average blood concentration of 4 mg. per 100 cc., the average reduction in power of accommodation was 1.50 D.

In 2 cases under sulfapyridine with an average blood concentration of 8 mg. per 100 cc., there was a 0.5 D reduction in accommodation in one and no reduction in the other. In 5 cases under sulfathiazole with an average blood concentration of 4.5 mg. per 100 cc., there was an average reduction in power of accommodation of 1.60 D.

Finally, Reynolds, Evans and Walsh[24] studied the effects on visual efficiency of small doses of sulfathiazole or sulfadiazine given for the prophylaxis of gonorrhea and chancroid. Eight subjects were given sulfathiazole and 8 sulfadiazine, 4 gm. in 24 hours at the rate of 1 gm. 4 times a day. Tests were made of the visual acuity, ocular muscle balance, abduction, adduction, fusion and convergence power, accommodation, depth perception, and size of the blind spots for color. The subjects were reëxamined 8 and 32 hours after the last dose. There was no significant change in the visual acuity. There was no alteration in the muscle balance at 20 feet; tests at close range showed a tendency to increased exophoria. Half of the subjects receiving sulfathiazole showed lowered abducting power at the end of 8, but not at the end of 32 hours. Those receiving sulfadiazine showed no change in abducting power. Consistent reduction in the power of adduction was found in the subjects receiving sulfathiazole at the end of 8 hours, but not at the end of 32 hours. Only 1 of the subjects receiving sulfadiazine showed lowered adducting power. There was reduced efficiency of convergence and fusion in half the cases at the end of 8 hours, but this was marked in only 2 of each group. At the end of 32 hours, there was more residual weakness of convergence in the sulfadiazine than in the sulfathiazole group. No consistent effect on the power of accommodation for far or near was noted. One subject who received sulfathiazole showed a definite spasm of accommodation. Depth perception was tested by means of the Howard-Dolman apparatus. The efficiency of depth perception was reduced in half the sulfathiazole and in all the sulfadiazine subjects at the end of 8 hours. In some of the sulfadiazine subjects the deficiency in depth perception was marked and was persistent at the end of 32 hours. The physiologic blind spots were measured on the tangent screen at 33 cm. with $\frac{1}{2}^\circ$ red and blue objects. Two subjects in each group showed a notable increase in the size of the blind spots or a constriction of the color fields. In 5 of the 8 subjects receiving sulfadiazine, the blind spots were found to be slightly abnormal. In the opinion of Reynolds, Evans and Walsh,[24] sulfathiazole and sulfadiazine administered in small "prophylactic" doses are "not without untoward transitory effect on visual efficiency" especially on the ocular muscle balance for near and on depth perception. They are inclined to think that sulfonamides should not be given to men who constantly require maximum visual efficiency, such as pilots, bombardiers, range finders, gun pointers, motorists, locomotive engineers, and precision workers.

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It has just come to my attention that R. W. Satterthwaite (J. Urol., **49**, 302, 1943) has reported 3 cases of conjunctivitis which occurred among 500 patients under treatment with sulfadiazine, along with other manifestations of toxicity. The conjunctivitis subsided within 3 days after the drug was stopped.

SURGERY

UNDER THE CHARGE OF

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BECAUSE of press of war work, there will be no Surgery Progress article in this issue.

BOOK REVIEWS AND NOTICES

BRUCELLOSIS IN MAN AND ANIMALS. By I. FOREST HUDDLESON, D.V.M., M.S., PH.D., Research Professor in Bacteriology, Michigan State College, and the following contributing authors: A. V. HARDY, M.S., M.D., DR.P.H., Associate Professor of Epidemiology, DeLamar Institute of Public Health, Columbia University Medical School Consultant, U. S. Public Health Service; J. E. DEBONO, M.D., M.R.C.P., Professor of Pharmacology and Therapeutics, Royal University of Malta; and WARD GILTNER, D.V.M., M.S., DR.P.H., Dean of Veterinary Division and Professor of Bacteriology, Michigan State College. Third Edition. Pp. 379; 42 figs. New York: The Commonwealth Fund, 1943. Price, $3.50.

WITH this revised edition, appearing 5 years after the first edition, the scope of the book has been broadened to include in addition to methods of laboratory diagnosis, the history, nature, diagnosis, treatment, and control of the disease. Considerable space is devoted to the bacteriology of the disease. "The value of new agents such as vaccines and drug therapy in the treatment of human brucellosis is analyzed, and recent information pertaining to the epidemiology and diagnosis of human brucellosis is set forth." There are appended 26 case reports illustrating different human clinical pictures, with frequent reference to these in the text. In view of some recent reports, it is noted that the relationship of brucellosis to Hodgkin's disease is probably coincidental, for the incidence of Hodgkin's disease in Malta is quite the opposite to the frequency of brucellosis. The authors have assembled in a handy volume practically all the pertinent knowledge related to this subject, and the book should enjoy widespread acceptance and use among bacteriologists, clinicians, veterinarians, and public health workers. M. T.

GYNECOLOGY. By LAWRENCE R. WHARTON, PH.B., M.D., Associate in Gynecology, The Johns Hopkins Medical School; Assistant Attending Gynecologist, The Johns Hopkins Hospital; Consultant in Gynecology, The Union Memorial Hospital, Hospital for Women of Maryland, Sinai Hospital and Church Home and Infirmary. Pp. 1006; 444 illus. Philadelphia and London: W. B. Saunders Company, 1943. Price, $10.00.

DR WHARTON is to be complimented on this production, which includes in one volume, the closely related subjects of gynecology and female urology. The most modern knowledge concerning operative and non-operative phases of gynecology and urology in the female is presented. His organization of the subject is logical and the text is written in very readable form.

The chapter on the anatomy of the pelvic organs is presented with excellent illustrations and a minimum of the usual confusing description. Embryologic development of the local organs is correlated with the occurrence of congenital malformations that are so commonly seen in the urogenital tract. In his discussion of the physiology of the sexual organs the author places particular stress on the recent knowledge concerning the endocrine relationships within the female.

Each organ—the vulva, vagina, cervix, uterus, Fallopian tube, ovary, umbilicus, and appendix—is discussed in detail as to the diseases and their diagnosis and methods of correction. The techniques of the operative treatments are illustrated step by step in large anatomic drawings. A consideration of the complications following gynecologic surgery is not neglected. Methods of prevention and treatment of these conditions are given.

The chapter on the relationships in symptomatology in diseases of the urinary and reproductive organs in women is a particularly helpful presentation for those charged with the responsibility of interpreting the complaints of the female. In this chapter the author emphasizes the bladder, ureteral, and renal symptoms that may be actually caused by gynecologic diseases, as well as abdominal and gynecologic symptoms that are actually due to diseases in the urinary organs. Important considerations in the differential diagnosis of the conditions are presented.

The section on urologic diseases in the female is made up of the following subjects: methods of urologic diagnosis, malformations of the urinary organs, urinary obstruction, non-specific urinary infection, urinary tract tuberculosis, urinary stone, urinary tumor, injury to the urinary tract, and chemotherapy in urinary tract infections. In this section particularly the author presents information that should be of interest not only to the gynecologist, but also the urologist.

Throughout the book each chapter is followed by an extensive bibliography offering excellent reference material for more detailed study of the subject matter.

As a reference book for the gynecologist and urologist, and as a text-book for the student this volume should fill a recognized need. L. L.

BEHAVIOR AND NEUROSIS: An Experimental Psychoanalytic Approach to PSYCHOBIOLOGIC PRINCIPLES. By JULES H. MASSERMAN, M.D., Assistant Professor of Psychiatry, University of Chicago, and Research Associate, Otho S. A. Sprague Memorial Institute, Chicago. Pp. 269; 7 plates. Chicago: The University of Chicago Press, 1943. Price, $3.00.

ANIMAL experimentation alone has convinced the author of the validity of showing that in a neurosis the fundamental psychodynamic factors are the same as in the more complex emotional disturbances of human beings. While a few dogs were used, most of the subjects were cats, as accidental stimuli affect them less, and their inhibitory functions being weaker, behavior shows less domination. Part I considers historical data and the results of laboratory experiments; Part II surveys pertinent literature on dynamic and experimental neuroses; Part III attempts the clinical application of such data. "To establish symbol-reactive and goal-directive patterns of behavior adaptation, animals were trained in an automatic apparatus to lift the lid of a box to secure food in response to one or more stimuli in various sensory modalities." Frustration and conflict, the result of mechanically induced fear and anxiety, are said to have so affected animal behavior that the subjects became "neurotic" or "psychotic." The bibliography exceeds more than 1000 titles.

N. Y.

THE INFECTIOUS DISEASES OF DOMESTIC ANIMALS. By WILLIAM ARTHUR HAGEN, D.V.M., D.Sc., Professor of Bacteriology and Dean of the Faculty, New York State Veterinary College, Cornell University. Pp. 665; 145 illus. Ithaca, N. Y.: Comstock Publishing Company, 1943. Price, $6.00.

THIS is a well-integrated and entirely adequate account of the host of infectious diseases to which domesticated mammals and birds are subject, of the specific microörganisms involved and of available methods of diagnosis and control. The introductory section of the book is a consideration of the general aspects of infection and disease production by microörganisms, and of the nature and development of the immune response, with a brief review of allergic conditions and of iso antibodies. Discussion of groups of microörganisms is arranged under the following section headings: Pathogenic Bacteria, Bacteria-like Pathogenic Organisms of Uncertain Classification, *i. e.*, Spirochetes, Rickettsiæ and Pleuro-pneumonia, Pathogenic Fungi, Pathogenic Protozoa,

and Viruses. For each of these groups or, where justified, for individual organisms, consideration follows the general pattern: morphology, reactions in culture, natural habitat, pathogenicity and types of disease in susceptible hosts, diagnostic and control methods, immune response, and, where appropriate, relation to disease of man. Each chapter and many chapter subdivisions have short lists of well-chosen references appended. This is not a textbook of bacteriology in the usual sense, but combines the several aspects of infectious disease to form one that seems a well-worthwhile addition to the texts which have been available in this field. H. R.

BEHIND THE SULFA DRUGS—A SHORT HISTORY OF CHEMOTHERAPY. By IAGO GALDSTON, M.D., with a Preface by PERRIN H. LONG, M.D., Associate Professor of Medicine, Johns Hopkins University. Pp. 174. New York: D. Appleton-Century Company, Inc., 1943. Price, $2.00.

THE author has described in non-technical language the development of chemical weapons against infectious diseases from ancient times to the present day. An appropriately generous portion of the book is devoted to the story of Paul Ehrlich and of his influence for and against progress in this field. Of particular interest is the discussion of "The Doldrum Years" from the time of Ehrlich until 1935 when the discovery of sulfonamide therapy was announced. These were the years when sulfanilamide remained on the chemists' shelves and escaped recognition as a chemotherapeutic agent, while most investigators continued to be ineffectively preoccupied with the possibilities of synthetic dyes in treating infections. This is a very interesting book and will be enjoyed equally by those with specialized or general interest in medical science. J. L.

TRANSURETHRAL PROSTATECTOMY. By REED M. NESBIT, M.D., F.A.C.S., Associate Professor of Surgery, University of Michigan Medical School, in charge of the Section of Urology, Dept. of Surgery. With original drawings illustrating techniques by WILLIAM P. DIDUSCH. A Chapter on the Vascular Supply of the Prostate Gland by RUBIN H. FLOCKS, M.D. Pp. 201; 62 plates. Springfield, Ill.: Charles C Thomas, 1943. Price, $7.50.

DR. NESBIT is recognized as one of the experts in the field of prostatic resection. His book records, in an excellent manner, his technique of surgery and methods of patient care.

The first chapter on arterial distribution within the prostate is based on the original work of Dr. Rubin H. Flocks. A knowledge of the circulation of the prostate is basic in the technique of prostatic resection. There is a chapter on the history of the development of the resectoscope.

This book should be of great value to anyone doing prostatic surgery as well as to anyone concerned with the care of prostatic patients. L. L.

SOCIAL INFLUENCES AFFECTING THE BEHAVIOR OF YOUNG CHILDREN. By RUTH PEARSON KOSHUK. Monographs of the Society for Research in Child Development, Vol. VI, No. 2 (serial No. 28). Pp. 71. Published by the Society for Research in Child Development, National Research Council, Washington, D. C., 1941. Price, $1.00.

THIS is a carefully organized survey of the significant research publications since 1925, dealing with the behavior of young children as influenced by social factors. The divergent points of view in this branch of "social psychiatry" are presented thoughtfully and with fairness. An extensive bibliography is appended. I. W.

THE THERAPY OF THE NEUROSES AND PSYCHOSES. By SAMUEL HENRY KRAINES, M.D., Associate in Psychiatry, University of Illinois, College of Medicine; Assistant State Alienist, State of Illinois; Diplomate of American Board of Psychiatry and Neurology; Captain, United States Medical Corps. Pp. 567; 6 figs. Second ed. Philadelphia: Lea & Febiger, 1943. Price, $5.50.

CAPTAIN KRAINES is at present a military lecturer on Psychiatric Orientation, at Fort Hood, Texas. This book of 20 chapters, written in excellent English, designed for the student and general practitioner, is a thoroughly revised second edition within 2 years. The space previously allotted to Psychoanalysis and Related Schools is reduced. Schizophrenia is discussed more fully, and there are additional chapters on Shock Therapy, the Organic Psychoses, Psychiatric Interview Techniques, and the Neuropsychiatric States Induced by War.

During the present wartime period, neuroses occur among the civil population because of; "(1) fear of personal attack or injury, (2) threat of economic insufficiency, (3) dangers of inadequate food supply . . . ., (4) threat of separation of family, and (5) disturbance of a routine manner of living. . . . ." Under Neuropsychiatric States in Military Service, the following are some of the topics discussed: such states among drafted men before army admission, and among military forces not in actual combat; such states under combat conditions; prophylactic therapy; tension symptoms; symbolic symptoms; therapy of acute neuropsychiatric states; shell shock; front line therapy; group psychotherapy; therapy in neuropsychiatric centers; individual therapy; desensitization; group hypnosis; socialization; and the return to active combat of those soldiers already cured.

Many of the socio-psycho-biologic problems of present-day civil and military life are ably discussed here. Over 200 illustrative cases are used, and there is an exceptionally comprehensive index of more than 35 pages. The writer's purpose of providing a practical understanding of neuropsychiatric states, with concrete suggestions on treatment, is sustained throughout.

N. Y.

AS THE TWIG IS BENT. By LESLIE B. HOHMAN, M.D., Associate in Psychiatry, Johns Hopkins Medical School; Assistant Visiting Psychiatrist to the Johns Hopkins Hospital. Pp. 291. New York: The Macmillan Company, 1943. Price, $2.50.

THE most striking point in this delightful book is its plain, common sense approach to the problem of raising children to be well-adjusted adults. Seven reprintings of this first edition testify to its popularity. Dr. Hohman is, without doubt, well versed in his field, as is evidenced by the wide variety of experiences he has had in dealing with children.

The style of his writing is simple, clear and essentially conversational. Case studies are not set down in the clinical style so often associated with a technical book; but they are used to illustrate a point the author is making at that particular minute—set forth in a few paragraphs and applied directly to the point in question. Problems that are discussed in the book are those that arise with a child's progressing age; and yet in many instances these problems have their roots in or near the child's infancy.

The book gives the reader a feeling of confidence that he, the reader, could be almost an exemplary parent if he made up his mind to spend the same amount of time, thought, interest in and preparation on becoming and being a parent as he does for a career, that he could be as successful in one as in the other—with the same amount of satisfaction in a job well done. Changed conditions in modern societal life have brought to the attention of the lay public the problems arising in marriage. So, too, we are recognizing the problems of modern parenthood and are striving to do something about them.

The Reviewer recommends this book highly to all those who are about to

become parents and to those who already are parents. One reading is not enough, for this is the type of book one will want to refer to many times to reassure himself that the course he is trying to steer is the best for all concerned.

E. F.

CIVILIAN HEALTH IN WARTIME. By FRANCIS R. DIEUAIDE, M.D., Associate Professor of Medicine, Harvard Medical School, Massachusetts General Hospital. Pp. 328; several tables. Cambridge, Mass.: Harvard University Press, 1942. Price, $2.50.

"This book represents an attempt to provide the general reader with a statement, in broad but definite outline, of the varied aspects of health in the United States in relation to the war. The point of view is that of positive health. Hence diseases are not described; nor are methods of treatment presented . . . . The aim of our enemies is to destroy our health, but through our health we shall defeat them."

This quotation is from the preface and indicates the author's serious purpose. The book is in readable though scholarly style, contains an abundance of well-chosen illustrations to emphasize the many points that are made, and discusses community and mental health problems as well as those of individual health. Nutrition, diet, safety from infectious disease, shelter and raiment, mother and child, geriatrics, occupation, recreation, medical and nursing services, mental calm and vigor and morale are given detailed discussion. The non-professional student of social science and current world problems who seeks an understanding of America's medical resources and attitudes in these critical years will find here a well organized, panoramic presentation free from subjective bias.

I. W.

DIE APPETITLOSIGKEIT IM KINDESALTER. By JULIUS SURANYI, Obenarzt am Stefanie Kinderspital (1940). Verlag von S. Karger in Basel (Switzerland). Pp. 128. For U. S. A.: New York: Nordeman Publishing Company, Dept. S. Karger, 215 Fourth Avenue. Price, S.fr. 6.

THIS monograph is a well-conceived and thoughtful survey of the problem of anorexia in infancy and childhood. The author's classification of the types of anorexia and of the types of children with the complaint is well done. Fully aware that loss of appetite is at best a symptom, the author outlines systematically the methods for studying the infant and child with anorexia. This study outline is an excellent, brief review of pediatric gastro-enterologic diagnostic procedure.

There is a detailed analysis of the somatic and psychologic factors responsible for acute or chronic loss of appetite. The discussion of the somatic factors is thorough and is cognizant of recent developments, *e. g.*, the rôle of infestation with *Giardia lamblia* as a disturbing influence. The section dealing with the psychologic aspects of anorexia is equally thorough, but the inclusion of a great deal of abstract speculative discussion does not make for clarity.

The therapeutic recommendations are sound and in accord with current pediatric and psychiatric procedure.

This monograph should be of interest to pediatricians and to psychiatrists concerned with child guidance.

M. R.

ADVANCES IN PEDIATRICS. Edited by ADOLPH G. DESANCTIS, M.D., New York Post-Graduate Medical School and Hospital, Columbia University, N. Y., and several associate editors. Pp. 306; many figs., plates and tables. New York: Interscience Publishers, Inc., 1942. Price, $4.50.

EACH chapter in this auspicious first volume of a projected annual series deals with some pediatric subject which has seen constructive progress within

the past few years. The contents comprise 9 short "personalized monographs," on Toxoplasmosis (Sabin); Virus Diseases (Hodes); Chemotherapy (Carey); Electroencephalography (Brill); Vitamin K in the Newborn Period (Poncher); Surgery of Persistent Ductus Arteriosus (Gross); The Premature Infant (Tow); Tuberculosis (Nelson); Endocrinology (Gordon), together with 9 "abstracts of some other advances in pediatrics" (DeSanctis and Pittinos). The authors of the major chapters are well known for special studies within their respective fields.

The quality of every article is excellent, and the style lucid and easy to read. This book is of importance not alone to specialists in pediatrics, who will find therein well-organized formulations of authoritative current points of view. It can be highly recommended as useful both to alert general practitioners who want to keep abreast of advances in this branch of medicine, and to internists and other specialists who desire an understanding of the early stages of some of the long-standing disorders of adult patients which often date back to childhood years. I. W.

MANUAL OF INDUSTRIAL HYGIENE AND MEDICAL SERVICE IN WAR INDUSTRIES. Edited by WILLIAM M. GAFAFER, D.Sc. Issued under the Auspices of the Committee on Industrial Medicine of the Division of Medical Sciences of the National Research Council. Prepared by the Division of Industrial Hygiene, National Institute of Health, U. S. Public Health Service. A composite book with 16 contributors. Pp. 508; 20 illus. Philadelphia and London: W. B. Saunders Company, 1943. Price, $3.00.

THIS excellent manual, as its name implies, is dedicated to industrial hygiene and medical services in war industries. The contributors, whose painstaking work has made this book possible, have been chosen because of their outstanding ability in the subject which they present.

The volume is presented in 3 parts: Part 1: Organization and Operation of Facilities, contains the following chapters: War's Influence on Industrial Hygiene; Plant Medical Facilities; Organization of Plant Medical Department; Medical, Nursing, and Dental Services; Organization of Plant Emergency Services; and Available Services in Industrial Hygiene. Part 2: Prevention and Control of Disease in Industry, is in 2 sections: Section 1 covers the problem of Occupational Dermatoses; Medical Control of Respiratory Diseases; Venereal Disease Control, Industrial Psychiatry, Health Education, Fatigue and Nutrition. Section 2 covers Community and Plant Sanitation, Illumination, Noise, and Radiant Energy, Heating, Ventilation and Air Conditioning. Part 3: The Manpower Problem, discusses the subject of maximum use of manpower, Women in Industry and Absenteeism. Appropriate bibliographic references accompany each chapter.

This book is most timely and is seriously recommended to all physicians, hygienists, safety men, and nurses engaged in industrial work. R. K.

THE HEARING OF SCHOOL CHILDREN: A STATISTICAL STUDY OF AUDIOMETRIC AND CLINICAL RECORDS. By A. CIOCCO and C. E. PALMER, Division of Public Health Methods, National Institute of Health, Bethesda, Md. Monographs of the Society for Research in Child Development, Vol. VI, No. 3 (serial No. 29). Pp. 77; 54 charts and figs. Published by Society for Research in Child Development, National Research Council, Washington, D. C., 1941. Price, $1.00.

THIS technical report summarizes an investigation by the U. S. Public Health Service, on hearing in childhood. In 1931, some 14,000 Washington, D. C., school children were given preliminary tests with a phonograph audiometer. Later, over 1400 of these were reëxamined, some several times, with the more reliable pure-tone instrument, thus acquiring a number of significant

observations. Audiometric curves in childhood were found to assume the same characteristics as in adults. There was a strong tendency for the two ears of the same child to give the same type of audiometric curve. The incidence of high tone loss increased with advancing age and was greater in boys than in girls. Hearing impairment was often associated with past or present inflammatory lesions of the tympanic membranes, but bore no relationship to the condition of nose and tonsils as observed at the time of the examination. From these data the authors conclude that about 5% of America's school children (close to 1½ millions) possess impairment of hearing in one or both ears sufficiently marked to become a handicap. Another fraction of equal size is estimated to suffer from high tone loss, a defect which does not interfere with daily life, though in certain situations it may be noticeable and annoying. By proper testing incipient deafness can be detected in its early subclinical stages. "It is only too obvious, therefore, that even a small loss of acuity cannot be disregarded if successful results are to be hoped for in preventing deafness; especially since very few ears with impaired hearing appear to improve when the impairment has reached the level here defined as marked." I. W.

A Study of Endometriosis, Endosalpingiosis, Endocervicosis and Peritoneo-ovarian Sclerosis. A Clinical and Pathological Study. By James R. Goodall, M.D., D.Sc., Formerly Professor of Gynecology and Obstetrics, McGill University. Pp. 140; 19 figs. Philadelphia: J. B. Lippincott Company, 1943. Price, $5.50.

Goodall has brought together under one cover all essential knowledge dealing with the whole subject of endometriosis, the first time that this has been done. It includes several chapters dealing with previously unpublished work in the field. The history of the subject is reviewed. The volume has a decidedly clinical slant; it deals with the question of diagnosis, records the latest developments in research, indicates how the condition spreads and speculates upon its causation. There are 13 illustrations in black and white and 17 subjects in full color. The observations appear to be largely those of the author, and the list of references relatively short. The book is one which should be in the library of every gynecologist, and might well be consulted by medical students during their clinical years when studying gynecology. D. M.

The Chemistry of Natural Coloring Matters. The Constitutions, Properties, and Biological Relations of the Important Natural Pigments. By Fritz Mayer, Ph.D., Formerly Professor of Chemistry in the University of Frankfort-on-Main. Translated and revised (from the third and last German edition) by A. H. Cook, Ph.D., Department of Chemistry, Imperial College of Science, London). American Chemical Society Monograph No. 89. Pp. 354. New York: Reinhold Publishing Corporation, 1943. Price, $10.00.

This volume is an addition to the growing number of authoritative scientific monographs published under the sponsorship of the American Chemical Society. It is an excellent compilation of data, almost exclusively of an organic chemical nature, upon several hundred pigments of plant and animal origin. The book is divided into 5 chapters, each of which embraces pigment derivatives belonging, from the standpoint of structure, to a different chemical class.

The chapters upon "Polyene Pigments" and "Heterocyclic Nitrogen Atoms" embrace commonly met substances, such as the carotenoids (including vitamin A), porphyrin derivatives of hemoglobin and chlorophyll, pterins, and flavins. The larger part of the text, however, is devoted to less well-known pigments, discussed mainly under the heads of "Carbocyclic Compounds" and "Heterocyclic Compounds."

The book is published posthumously. Professor Cook, the translator-editor, records in a preface that Professor Fritz Mayer "died in July, 1940, in circumstances which, it need only be said, were as sad as any imaginable."

The Reviewer recommends the book as a useful reference work in its field, covered comprehensively and withal briefly. D. D.

MILITARY SURGICAL MANUALS. V. BURNS, SHOCK, WOUND HEALING AND VASCULAR INJURIES. Prepared under the Auspices of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. Pp. 272; 81 figs. Philadelphia and London: W. B. Saunders Company, 1943. Price, $2.50.

MANY distinguished American surgeons have collaborated to produce this book. Each of the subjects named in the title has been subdivided into many chapters, and each chapter summarizes the views and experiences of one or several men on the topic in question. It is presumed that all statements have been reviewed editorially by the respective subcommittees of the Committee on Surgery. The book was designed to familiarize doctors being called into military service with current concepts in certain provinces of military surgery, and one can say that this purpose is well fulfilled. An abundance of useful non-controversial information is contained herein. Some matters receive more emphasis than others of equal or greater importance, for example, more space is allotted to the arteritis:scalenus syndrome than to the treatment of varicose veins by injection, but such lack of evenness can be excused by the multiplicity of authorship. The illustrations are excellent. I. W.

MEDICAL JURISPRUDENCE AND TOXICOLOGY. By JOHN GLAISTER, M.D., D.Sc., Fellow of the Royal Faculty of Physicians and Surgeons, Glasgow; of the Inner Temple, Barrister-at-Law, etc., Regius Professor of Forensic Medicine, University of Glasgow; Formerly Professor of Forensic Medicine, University of Egypt, Cairo; and Medico-Legal Consultant to the Egyptian Government. Pp. 671; 132 illus. Seventh ed. Baltimore: The Williams & Wilkins Company, 1942. Price, $8.00.

IN its 7th edition, the form of this standard text has been recast and it has been for the most part rewritten; many illustrative cases are new. In the section on Toxicology some new poisons have been added, notably the sulfa drugs (sulfanilamide, sulfapyridine and sulfathiazole); and the tests for the identification of poisons have been limited, except in a very few instances, to qualitative tests, in recognition of the specialized character of quantitative toxicologic procedures. A chapter on war gases and the effects of blast has been added, and a special appendix on procedures in deaths following air raids.

The book is very well illustrated and indexed. The laws cited are limited to those of England and Scotland, and in this one respect the book does not meet completely the needs of the American reader. E. W.

NEUROLOGY. By ROY R. GRINKER, M.D., Chairman, The Department of Neuropsychiatry of the Michael Reese Hospital, Chicago. Third ed. Pp. 1136; 416 figs. Springfield, Ill., and Baltimore, Md.: Charles C Thomas, 1943. Price, $6.50.

THE new edition of this book has been enlarged, largely rewritten and revised by the author with the assistance of Norman A. Levy, M.D., Associate Neuropsychiatrist of the Michael Reese Hospital. An excellent chapter on Brain Tumors has been contributed by Paul C. Bucy, M.D., Professor of Neurology and Neurological Surgery of the University of Illinois.

The original plan of a biologically oriented neurology has been maintained without sacrificing practical diagnosis and therapy. The 22 chapters of the book cover practically the entire field of Neurology. Many illustrations of electroencephalography and pneumoencephalography greatly clarify the text. Advances in the study of encephalitis and the function of the hypothalmus are well covered. The subject of aphasia is too briefly described but adequate references are given. Separate chapters on the epilepsies and headache include the most recent ideas as to etiology and treatment. Trauma of the central nervous system is thoroughly considered, including herniation of the nucleus pulposus; but the medico-legal aspect of the subject is unfortunately omitted. The book is well indexed and contains many new illustrations and valuable tables. Recent literature has been added without deletion of the most important older literature. A change of typography adds to the ease of reading. This excellent textbook of Neurology is highly recommended. A. S.

Clinical Significance of the Blood in Tuberculosis. By Gulli Lindh Muller, M.D., Pathologist and Director of Laboratory, New England Hospital for Women and Children, Boston; Formerly Pathologist, Rutland State Sanatorium, Rutland, Mass. Pp. 516; 52 tables, 19 charts. New York: The Commonwealth Fund, 1943. Price, $3.50.

The importance of the blood picture in tuberculosis is not generally appreciated. Dr. Muller's book is a complete critical review and correlation of the literature on the subject, combined with an analysis of 6819 blood examinations done on 1000 patients over 5 years. The text is well organized (including a complete Bibliography) and the contents are divided into 6 parts: the first being a summary of the physiology of the hemopoietic system and its response to the tubercle bacilli; Part 2 discusses the changes and response of the various cellular elements both qualitatively and quantitatively in tuberculosis. Part 3 dealing with the sedimentation rate is a complete review of the subject, stressing the importance of this test in tuberculosis and presenting a new table for corrections. Parts 4 and 5 compare clinical and hematologic data in various stages and types of diseases, methods of treatment and complications, demonstrating that the blood picture and sedimentation rate give information, not otherwise obtainable, concerning the status of the disease and its prognosis. Part 6 gives in detail her methods. This book fills a very definite need in the field of tuberculosis and should be read by every phthisiologist. D. C.

Essentials of Industrial Health. By C. O. Sappington, M.D., Dr.P.H., Consulting Industrial Hygienist, President, Central States Society of Industrial Medicine and Surgery; Editor of "Industrial Medicine." Pp. 626; 63 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, $6.50.

This text is the most recent addition to a long list of outstanding contributions, by one of the foremost medical authorities in the industrial field. It is a comprehensive undertaking and presents a panorama of all the phases of industrial health, designed particularly to aid those already in this type work, and especially to familarize those, either new in the field or contemplating such a change.

The book makes for quick and easy reading, and is presented in 3 parts. The first, Industrial Health Administration, ranges from an excellent chapter on the history of industrial medicine, through legislative background to rehabilitation of workers. The second third discusses Industrial Hygiene and Toxicology with chapters on Exposures, Plant Survey, Plant Sanitation and Hygiene, Personal Hygiene. The final third is on Industrial Medicine and Traumatic Surgery, covering the Worker, Accidents, Occupational and Non-occupational Conditions, plus Workmen's Compensation and Rehabilitation.

In the Appendix there is technical material of great value, plus a well-selected Bibliography.

This book is heartily recommended as a ready reference book for all interested in this most important field. R. K.

A GUIDE TO PRACTICAL NUTRITION: A series of articles on nutrition, sponsored by the Committee on Nutrition and Deficiency Diseases of The Philadelphia County Medical Society. Edited for the Committee by MICHAEL G. WOHL, M.D., and JOHN W. WILLARD, M.D. Pp. 98. The Philadelphia County Medical Society, 1943.

As stated in the introduction by Dr. Morris Fishbein, this committee "rendered a distinct service by publishing in *Philadelphia Medicine* some of the basic facts in the field concerned. This material was so favorably received that it is now being extended with the aid of John Wyeth and Bro., Inc., to a much larger audience." It consists of concise but adequate chapters on most of the clinical aspects of nutrition with many suggested diets. In the appendix is a vitamin chart and a table of food composition, weights, and measures. M. T.

WAYS OF THE WEATHER. By W. J. HUMPHREYS, A.B., C.E., PH.D., SC.D., Meteorological Physicist (Retired, Collaborator), U. S. Weather Bureau. Pp. 400; 75 illus. Lancaster, Pa.: Jaques Cattell Press, 1942. Price, $4.00.

THIS volume is one of a "humanizing science series" written for the lay public. It is written in a rather simple manner and succeeds in giving the reader a good, basic understanding of the weather and the factors involved in producing it. In 16 chapters the author presents in satisfactory detail a rather complete treatise from the origin and composition of the atmosphere and the rôle played by each of its constituents in the production of wind, precipitation, temperature, pressure, and so forth, to the application and influence of weather both bad or good on our lives. This book not only is enjoyable to read, but also substantially enhances our knowledge of the most talked about peacetime subject, the weather. M. T.

NEW BOOKS

A Handbook of Medical Library Practice. Editor, JANET DOE. Compiled by a Committee of the Medical Library Association. Based on a Preliminary Manuscript by M. IRENE JONES. Pp. 609. Chicago: American Library Association, 1943. Price, $5.00.

Whooping Cough. By JOSEPH H. LAPIN, B.CHEM., M.D., Adjunct Pediatrician, Bronx Hospital, Associate in Contagion, Riverside Hospital for Contagious Diseases, New York. Pp. 237; 25 tables; 40 illus. Springfield, Ill.: Charles C Thomas, 1943. Price, $4.50.

Diagnosis of Uterine Cancer by the Vaginal Smear. By GEORGE N. PAPANICOLAOU, M.D., PH.D., Department of Anatomy, Cornell University Medical College; and HERBERT F. TRAUT, M.D., Department of Obstetrics and Gynecology, Cornell University Medical College and the New York Hospital. Pp. 47; 11 color plates. New York: The Commonwealth Fund, 1943. Price, $5.00.

Pictorial Handbook of Fracture Treatment. By EDWARD L. COMPERE, M.D., F.A.C.S., Associate Professor of Surgery, Northwestern University Medical School; Chairman, Department of Orthopædic Surgery, Wesley Memorial Hospital; Consulting Orthopædic Surgeon, Chicago Memorial Hospital; and SAM W. BANKS, M.D., Associate in Surgery, Northwestern University Medical School; Attending Orthopædic Surgeon, Chicago Memorial Hospital. Pp. 351; 171 figs. Chicago: The Year Book Publishers, Inc., 1943. Price, $4.25.

The Role of Nutritional Deficiency in Nervous and Mental Disease. Vol. XXII. Proceedings of the Association Dec. 19 and 20, 1941, New York. Editorial Board: STANLEY COBB, M.D., Chairman, EDWIN F. GILDEA, M.D., HARRY M. ZIMMERMAN, M.D. Pp. 215; 23 illus., 8 tables. Baltimore: Williams & Wilkins Company, 1943. Price, $4.00.

Human Neuroanatomy. By OLIVER S. STRONG, Formerly Professor of Neurology and Neurohistology, College of Physicians and Surgeons, Columbia University; and ADOLPH ELWYN, Associate Professor of Neuroanatomy, College of Physicians and Surgeons, Columbia University. Pp. 417; 320 figs. Baltimore: Williams & Wilkins Company, 1943. Price, $6.00.

Air-borne Infection. By DWIGHT O'HARA, M.D., Professor of Preventive Medicine, Tufts College Medical School; Visiting Physician, Boston City Hospital; Physician-in-Chief, Waltham Hospital. Pp. 114; several charts and tables. New York: The Commonwealth Fund, 1943. Price, $1.50.

Textbook of Anatomy and Physiology for Nurses. By CARL C. FRANCIS, A.B., M.D., Senior Instructor in Anatomy, Department of Anatomy, Western Reserve University, Cleveland; G. CLINTON KNOWLTON, PH.D., Assistant Professor of Physiology, College of Medicine, State University of Iowa; and W. W. TUTTLE, PH.D., Professor of Physiology, College of Medicine, State University of Iowa. Pp. 586; 338 illus. (38 color plates). St. Louis: C. V. Mosby Company, 1943. Price, $3.50.

Skin Grafting of Burns. By JAMES B. BROWN, M.D., LT.-COL., M.C., U. S. A., Senior Consultant in Plastic and Maxillo-facial Injuries and Burns, E. T. O., U. S. A., Associate Professor of Surgery, Washington University, St. Louis; and FRANK MCDOWELL, M.D., Assistant in Clinical Surgery, Washington University. Pp. 204; 131 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, $5.00.

Your Arthritis: What You Can Do About It. By ALFRED E. PHELPS, M.D., Introduction by R. GARFIELD SNYDER, M.D. Illustrations by JAMES MACDONALD. Pp. 192; 12 illus. New York: William Morrow & Co., 1943. Price, $2.00.

Essentials of Syphilology. By RUDOLPH H. KAMPMEIER, A.B., M.D., Associate Professor of Medicine, Vanderbilt University School of Medicine; in Charge of the Syphilis Clinic and Visiting Physician to Vanderbilt University Hospital. With chapters by ALVIN E. KELLER, M.D., and J. CYRIL PETERSON, M.D. Pp. 518; 87 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, $5.00.

The Common Form of Niacin Amide Deficiency Disease: Aniacinamidosis. By WILLIAM KAUFMAN, PH.D., M.D. Pp. 62. Bridgeport, Conn.: Yale University Press, 1943. Price, $3.00.

NEW EDITIONS

The Practice of Refraction. By SIR STEWART DUKE-ELDER, M.A., D.SC. (ST. AND.), PH.D. (LOND.), M.D., CH.B., F.R.C.S., Surgeon-Oculist to H. M. The King; Ophthalmic Surgeon and Lecturer in Ophthalmology. St. George's Hospital. Fourth ed. Pp. 328; 183 illus. Philadelphia: The Blakiston Company, 1943. Price, $4.50.

Injuries of the Skull, Brain and Spinal Cord. Edited by SAMUEL BROCK, M.D., New York University. Second ed. Pp. 616; 78 figs. Baltimore: Williams & Wilkins Company, 1943. Price, $7.00.

The Therapy of the Neuroses and Psychoses. By SAMUEL H. KRAINES, M.D., Associate in Psychiatry, University of Illinois, College of Medicine; Assistant State Alienist, State of Illinois; Diplomate of American Board of Psychiatry and Neurology; Captain, M.C., U.S.A. Second ed. Pp. 567; 5 figs. Philadelphia: Lea & Febiger, 1943. Price, $5.50.

Pye's Surgical Handicraft. Edited by HAMILTON BAILEY, F.R.C.S. (ENG.), Surgeon, Royal Northern Hospital, London; Surgeon and Urologist, Essex County Council; Surgeon, Italian Hospital; Consulting Surgeon, Clacton Hospital and the County Hospital, Chatham; External Examiner in Surgery, University of Bristol. Thirteenth ed. Pp. 536; 534 illus. Baltimore: Williams & Wilkins Company, 1942. Price, $6.00.

Methods for Diagnostic Bacteriology. By ISABELLE G. SCHAUB, A.B., Instructor in Bacteriology, Dept. of Pathology and Bacteriology, The Johns Hopkins University School of Medicine; Bacteriologist in Charge of the Diagnostic Bacteriological Laboratory of the Woman's Clinic, The Johns Hopkins Hospital; Instructor in Bacteriology in the Nurses Training School; and M. KATHLEEN FOLEY, A.B., Bacteriologist in Charge of the Diagnostic Bacteriological Laboratory of the Medical Clinic, The Johns Hopkins Hospital. Second ed. Pp. 430. St. Louis: C. V. Mosby Company, 1943. Price, $3.50.

ERRATUM

In the article on "Studies on the Hemorrhagic Agent 3,3′-Methylenebis (4-Hydroxycoumarin): Part III" by James B. Bingham, Ovid O. Meyer, and Beryl Howard, appearing in the April issue of this Journal (**205**, 587–594, 1943), the authors request that the following errors in their abstract of Case 1 be corrected: On page 588, 9 lines from bottom, "subcutaneously" should read "intravenously"; and on page 589, line 2 should read "no question that continuous intravenous is preferable to intermittent administration of heparin."

E. B. KRUMBHAAR.

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